THE RESPONSE OF ARTEMISIA TILESII TO SIMULATED ACID PRECIPITATION

by

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INTRODUCTION

Rain and mist have many effects upon plants beyond providing water for growth. These include inputs of nutrients and leaching of minerals, amino acids, carbohydrates and growth regulators, thus influencing the initiation and development of roots, seed dormancy, flowering and fruit set. The integral role that precipitation plays in these important aspects of plant growth and development justifies the serious concerns that have been expressed by scientists over the significant changes that have occurred in the composition of wet deposition over the last 30 years. Reference to the acidification of precipitation, produced by long range transport of air pollutants, first appeared with a publication by Odén in 1968 outlining the spread and extent of the acidity problem in northern Europe. Since that time a substantial volume of literature on the wet deposition of pollutants and the effects of those precipitation-deposited pollutants on vegetation and soils has appeared.

The present study was undertaken to determine the response of Artemisia tilesii to simulated acid rain. This species survives, and shows very little visible injury, at the Smoking Hills in the Northwest Territories where burning shale cliffs produce high levels of SO_2 and acid aerosol. Before discussing the specific objectives of this research project it is useful to outline the extent of the acidic precipitation problem in Ontario as a basis for investigating effects of acidity in precipitation. A review of the knowledge that has been accumulated on the effects of acidic precipitation on vegetation, mostly from studies involving application of simulated acid rain, will follow. The intent of this review is to point out common conclusions as well as inconsistencies in the literature to date and to indicate some areas where research is lacking or further study is necessary.

I. PH OF PRECIPITATION IN ONTARIO

Atmospheric monitoring networks in Europe and North America show that the areas affected by acidic precipitation have spread in Europe and have been found to occur and are spreading in eastern North America. Data from the Acidic Precipitation in Ontario Study (APIOS) event deposition network for 1981 (Table 1) shows that the mean pH of precipitation over large portions of Ontario was less than 4.2. Although the data are for a single year, there is evidence of at least a slight seasonal trend in the pH pattern for Ontario, with the lowest values being recorded during the spring and summer months (Table 2). Data for the Longwoods station, located in the southwestern Ontario agricultural belt depicted this trend more strongly than the other stations. Here the mean monthly pH values were as low as 3.74, during the spring and summer months when most plants are actively growing. Raynor and Hayes (1978) have also found that the pH of rainwater is lowest in the spring and summer at Brookhaven, New York.

The input of hydrogen ions in acidic rain is 10 to 400 times greater than the quantities of hydrogen ions in "unpolluted" rainfall, which has a theoretical pH of 5.6. The cause of the increased acidity of precipitation is attributed to sulphur and nitrogen oxides, especially from high temperature combustion, from smelting and from coal-fired power plants, and is discussed in Likens and Bormann (1974), Galloway *et al* (1976), Likens *et al* (1976) and Vermeulen (1980). It is

Table 1. Concentration of free hydrogen ion and pH ranges for Ontario rainfall for the year of 1981. (Ministry of the Environment, W. Chan, personal communication)

	Concentration $(g\ l^{-1})$		
Station	Max.	Min.	Arithmetic mean
Dorset[a] (H^+)	4.27×10^{-4}	8.32×10^{-8}	7.04×10^{-5}
lab pH	7.08	3.37	4.15[b]
Longwoods[a] (H^+)	5.89×10^{-4}	2.00×10^{-8}	6.10×10^{-5}
lab pH	7.70	3.23	4.21[b]
Railton[a] (H^+)	4.79×10^{-4}	8.13×10^{-8}	7.50×10^{-5}
lab pH	7.09	3.32	4.12[b]

[a] The Dorset, Longwoods and Railton stations are located in central, southwestern and southeastern Ontario, respectively.

[b] Calculated from mean $[H^+]$

Table 2. Monthly free hydrogen concentration and pH values. (Ministry of the Environment, W. Chan, personal communication)

Month 1981	Dorset			Longwoods			Railton		
	N*	$[H^+]$† ±S.D.	pH	N*	[H]† ±S.D.	pH	N*	[H]† ±S.D.	pH
Jan	6	9.55×10^{-5} $\pm 7.18 \times 10^{-5}$	4.02	6	5.14×10^{-5} $\pm 5.90 \times 10^{-5}$	4.29	4	1.18×10^{-4} $\pm 0.48 \times 10^{-4}$	3.93
Feb	14	9.62×10^{-5} $\pm 6.44 \times 10^{-5}$	4.02	10	8.71×10^{-5} $\pm 11.4 \times 10^{-5}$	4.06	11	4.23×10^{-5} $\pm 2.83 \times 10^{-5}$	4.37
Mar	17	5.46×10^{-5} $\pm 5.12 \times 10^{-5}$	4.26	9	2.66×10^{-5} $\pm 5.35 \times 10^{-5}$	4.58	13	4.03×10^{-5} $\pm 7.36 \times 10^{-5}$	4.39
Apr	11	5.56×10^{-5} $\pm 3.62 \times 10^{-5}$	4.25	12	2.38×10^{-5} $\pm 3.39 \times 10^{-5}$	4.62	8	4.50×10^{-5} $\pm 2.29 \times 10^{-5}$	4.35
May	9	9.99×10^{-5} $\pm 8.90 \times 10^{-5}$	4.00	9	9.16×10^{-5} $\pm 7.54 \times 10^{-5}$	4.04	9	9.61×10^{-5} $\pm 5.60 \times 10^{-5}$	4.02
Jun	12	5.44×10^{-5} $\pm 3.88 \times 10^{-5}$	4.26	10	7.50×10^{-5} $\pm 4.77 \times 10^{-5}$	4.12	12	9.59×10^{-5} $\pm 8.17 \times 10^{-5}$	4.02
Jul	3	6.36×10^{-5} $\pm 6.03 \times 10^{-5}$	4.20	9	1.82×10^{-4} $\pm 1.80 \times 10^{-4}$	3.74	5	1.30×10^{-4} $\pm 1.97 \times 10^{-4}$	3.89
Aug	12	1.19×10^{-4} $\pm 1.14 \times 10^{-4}$	3.92	8	1.03×10^{-4} $\pm 0.68 \times 10^{-4}$	3.99	9	1.09×10^{-4} $\pm 0.47 \times 10^{-4}$	3.96
Sep	11	5.75×10^{-5} $\pm 5.02 \times 10^{-5}$	4.24	6	3.35×10^{-5} $\pm 3.30 \times 10^{-5}$	4.47	14	6.50×10^{-5} $\pm 6.24 \times 10^{-5}$	4.19
Oct	11	4.36×10^{-5} $\pm 3.70 \times 10^{-5}$	4.36	11	3.96×10^{-5} $\pm 3.81 \times 10^{-5}$	4.40	8	6.48×10^{-5} $\pm 4.79 \times 10^{-5}$	4.19
Nov	9	4.76×10^{-5} $\pm 3.20 \times 10^{-5}$	4.32	6	3.02×10^{-5} $\pm 3.08 \times 10^{-5}$	4.52	7	8.34×10^{-5} $\pm 6.79 \times 10^{-5}$	4.08
Dec	17	6.59×10^{-5} $\pm 4.64 \times 10^{-5}$	4.18	15	1.38×10^{-5} $\pm 2.02 \times 10^{-5}$	4.86	10	7.70×10^{-5} $\pm 4.02 \times 10^{-5}$	4.11

*N = number of rain events

†Arithmetic mean concentration (g l^{-1})

now generally recognized that the dry deposition of gases and particulates also contributes significantly to surface acidifying loads (Whelpdale, 1978,79; Lindberg et al, 1981a; Hicks, 1980). Lindberg et al (1981a, b) showed that interaction between moisture (dew, fog, rain) and previously dry-deposited particles on leaves resulted in concentrations of elements in solution orders of magnitude above concentrations in rain alone. Dry deposition must not be neglected in assessing the potential effects of acid rain.

Although the routine measurement of wet deposition in a geographic network is well established, it is only very recently that monitoring of acidity in precipitation has been conducted on individual rain events. From the point of view of assessing the direct biological effects on vegetation, average acidity values over a month do not give a very good picture of what the plant is actually experiencing on a daily basis. Yet, this can be of considerable importance. Data for one year (1981) were available in Ontario (W. Chan, M.O.E., personal communication), and three representative areas within the province were chosen to categorize the acidity in precipitation by the percentage of rainfalls within various pH ranges. Most rainfalls had pH levels between 3.75 and 4.5. Less than 1.5% of all rainfalls were below pH 3.5 (Table 3). A similar distribution of the pH of rainfalls was observed in the northeastern United States for the three years 1976 - 1979 (Evans et al, 1981c). Very low pH values (pH 2.1) have been reported for individual storms (Likens and Bormann, 1974), but these must be considered extremely rare. Direct foliar damage might well correlate best with the occurrence of the most acidic events, rather than with monthly means or totals.

Table 3. Percentage distribution of precipitation events in Ontario[a] for 1981 by pH (Ministry of the Environment, W. Chan, personal communication). Mean pH of all samples was 4.16.

pH interval	Frequency	Percentage of all events
3.00 - 3.24	1	0.29
3.25 - 3.49	4	1.17
3.50 - 3.74	23	6.70
3.75 - 3.99	52	15.16
4.00 - 4.24	82	23.91
4.25 - 4.49	77	22.45
4.50 - 4.74	33	9.62
4.75 - 4.99	20	5.83
5.00 - 5.24	8	2.33
5.25 - 5.49	8	2.33
5.50 - 5.74	10	2.91
5.75 - 5.99	5	1.46
6.00 - 6.24	8	2.33
6.25 - 6.49	6	1.75
6.50 - 6.74	2	0.58
6.75 - 7.74	5	1.46

[a] pH values for precipitation samples collected from the Longwoods, Dorset and Railton stations were chosen for this categorization because they are three representative areas (southwestern, central and southeastern) within Ontario.

II. EFFECTS OF ACID PRECIPITATION ON VEGETATION: LITERATURE REVIEW

A large number of studies have attempted to examine the effects that increases in the sulphate, nitrate and hydrogen ion concentration of rainfall may have on vegetation. While the damaging effect caused by acid rain to poorly buffered lakes and fish populations is well established (Gjessing *et al*, 1976; Leivestad *et al*, 1976; Harvey, 1980; Muniz and Leivestad, 1980), there are as yet no documented cases of damage to vegetation in the field directly caused by acid precipitation. Nevertheless, there is evidence from a growing number of studies, involving in most instances experimental applications of acidic rains to vegetation, that there are many potential effects that acid precipitation may have on sensitive plant species.

Effects of acidic precipitation on plant foliage:

Simulated acid rain has been shown to cause the development of necrotic lesions on the foliage of both coniferous and herbaceous species (Hindawi and Ratsch, 1974; Wood and Bormann, 1974; Gordon and Tourangeau, 1975; Shriner *et al*, 1975; Ferenbaugh, 1976; Abrahamsen *et al*, 1976; Jacobson and Leuken, 1977; Evans *et al*, 1977b; Shriner, 1978a; Evans *et al*, 1978; Evans and Curry, 1979; Evans, 1980; Lee *et al*, 1981). The threshold for the occurrence of foliar lesions is typically in the range of pH 3.0 to 3.2, but may vary from pH 2.0 for the very waxy leaves of citrus (Heagle *et al*, 1978) to 4.0 for some sensitive crop plants (Lee *et al*, 1981). Wettability of leaf surfaces is probably one of the most significant factors determining their sensitivity to injury from wet deposition of acidic substances. Waxy leaves apparently reduce the contact time for the acid solutions, thus accounting for the >400 fold increase in H ion concentration required to induce visible injury (Shriner, 1981). Cutin and waxes which impregnate the cell wall (Esau, 1977) probably also reduce penetration of acid solutions into cell walls and to the protoplast. Removal of leaf surface waxes has been reported to increase wettability of those surfaces (Martin and Juniper, 1970; Holloway, 1971). Critical studies examining the sensitivity of epiphytic lichens to acid rain have been lacking to date. Because lichens do not have a protective waxy cuticle, one would predict they might be highly sensitive to the wet deposition of pollutants. Epiphytic lichens are very sensitive to gaseous air pollutants and have been used as bioindicators of SO_2 pollution (Gilbert, 1965). It has recently been reported that simulated rain with a pH of 4.0 depressed the photosynthetic rate of the caribou lichen, Cladina stellaris, and lengthened the time required for full recovery of photosynthesis after wetting of dormant caribou lichens (Lechowicz, 1981).

A significant portion of the leaf area may exhibit lesions after exposure to simulated acid rain. For example, in a study of 28 crops the maximum percentage of leaf area injured at harvest was for radish, which had up to 25% of the leaf area damaged by acidic rain of pH 3.0 (Lee et al, 1981). Foliar injury sustained to swiss chard, mustard greens and spinach was severe enough to adversely affect their marketability. These experiments were conducted in exposure chambers in the field. However, in greenhouse studies where investigators have worked with the same species, visible injury has been found to occur at less acidic pH's in some instances (Jacobson, 1980). This may be explained by a greater hardening of leaf tissue under outdoor conditions. Lang et al (1978) have reported that erosion of cuticular surface waxes from leaves of willow oak (Quercus phellos) occurred as a result of exposure to simulated acidic rainfall of pH 3.2 for two months. More recently, Shriner (1980) and Shriner and Cowling (1980) discussed the potential effects of such weathering on the plant's ability to respond to a variety of external stresses, including plant pathogens, drought and gaseous air pollutants. In contrast, no effect of rainfall acidified down to pH 2.5 could be detected on the amount of wax on needles of two conifer species (spruce and lodgepole pine), as determined by extraction, weighing or examination by scanning electron microscopy (Horntvedt et al, 1980).

The amount of visible leaf injury is also influenced by tissue age. This can be seen in the work of Wood and Bormann (1974) and Evans et al (1977a), who reported young seedlings of birch and sunflower to be much more sensitive than older seedlings. Studies by Hindawi and Ratsch (1974) and Gordon and Tourangeau (1975) also show that actively growing tissue (cambium) is highly sensitive to injury, which led to the reduction of needle elongation in Scots pine, known as the "short-long needle syndrome". In general, leaves that are expanding rapidly, or have recently expanded are most sensitive to acid rain. Very small, immature and older leaves are more resistant (Evans et al, 1977a, 1978; Evans and Curry, 1979). The reasons for this are not known but probably relate to the development and hardening of the cuticle and to the vertical orientation of very young leaves.

Histological responses of vegetation to acidic precipitation have received limited attention, although Evans et al (1978) and Evans and Curry (1979) have shown that histological responses of leaves may influence their sensitivity to acid rain. These investigators have studied the histological responses to acid rain for a number of species and found that species in which hypertrophy (abnormal cell enlargement) and hyperplasia (abnormal cell division) occurred in mesophyll tissues had less foliar injury than species in which these responses did not occur. Cellular injury was observed to occur first in cells of the adaxial epidermis and affected internal tissues progressively during lesion development. More attention should be given to histological responses to acidic rain, since cellular damage may be a more sensitive indicator of acid rain injury occurring in natural situations.

Besides species susceptibility and stage of plant development, physical factors of the rainfall treatment (e.g. mist intensity, duration and frequency) are also important factors determining the threshold for foliar lesions. Jacobson and Leuken (1977) reported foliar injury to herbaceous species at pH 2.6 for a treatment duration of 1 minute, pH 3.0 or below for a total duration of 1.5 hours and pH 3.2 for a total duration of 3 hours. It is clear from the evidence available that a direct and useful comparison of results between different workers, who have inevitably used a variety of exposure periods, different frequencies of exposure, droplet sizes etc. may be difficult. Nevertheless, this provides a wide range of conditions under which vegetation has been exposed and from which we can evaluate the effects of a common pollutant. A number of common trends have appeared, which will add confidence to conclusions as to the manner in which acidic rainfall is affecting vegetation. For visible injury to foliage the general conclusions that can be drawn about the sensitivity of species are:

(1) dicotyledons in general are more sensitive than monocotyledons

(2) herbaceous species are the most sensitive, followed by deciduous woody species, followed by coniferous species

Effects of acidic precipitation on plant productivity

Research on the effects of acidic rain on agricultural crops has received considerable attention during the past several years in the United States. With simulated acidic precipitation at pH values between 3 and 4, both inhibitory and stimulatory effects on growth and yield, as well as no effects at all, have been obtained with a large variety of greenhouse- and field-grown crops. Lee *et al* (1981) exposed 28 crops to simulated sulphuric acid rain of pH 3.0, 3.5 and 4.0, and to a control rain of pH 5.6. The crops were grown in field chambers and marketable yield was compared at harvest. Marketable yield was inhibited for 5 crops (radish, beet, carrot, mustard greens and broccoli) and stimulated for 6 crops (tomato, green pepper, strawberry, alfalfa, orchard grass and timothy) at rain pH's between 3.0 and 4.0. No effects on productivity were observed for the other 17 crops. Perhaps most surprising was their finding that foliar injury was not generally correlated with effects on yield, as illustrated by carrots which sustained a 25% reduction in yield at pH 4.0 compared to control plants, yet had no visible foliar injury. Mohamed (1978) similarly found that at pH 4.0 yield of some crops was inhibited in the absence of visible injury to leaves.

Evans and Lewin (1981), working with greenhouse-grown soybeans and pinto beans, found differences in the response of the two crops to simulated acidic rain. Simulated acid rain of pH 3.1 and below, decreased the dry weight of seeds, leaves and stems of pinto beans. They attributed the decrease in the yield of seeds to both a decrease in the number of pods per plant and a decrease in the number of seeds per pod. In contrast, the seed yield of soybeans was increased by rain

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Several other experiments have been performed to assess effects of acidic rain on productivity of soybeans which have been grown under standard agronomic practices in the field (Irving and Miller, 1977,78,80; Jacobson et al, 1980; Evans et al, 1980). The performance of soybeans growing in plots to which artificial rainfalls of pH 4.0, 3.1, 2.7 and 2.3 were applied produced a small, but significant reduction in seed mass (2.6, 6.5, 11.4 and 8.5 % for the treatments respectively) when compared to soybeans receiving only ambient rainfall at Brookhaven, New York, with an average pH of approximately 4.0 over the period of this experiment (Evans et al, 1980). The decrease in yield was a direct reflection of the number of pods per plant since the number of seeds per pod and the dry mass of individual seeds did not differ among the experimental treatments. Seed protein contents were significantly lower ($P<0.05$) in plants exposed to simulated acidic rains at pH 4.0 and 3.1 compared with no additional rain treatments, while the content of non-structural carbohydrates was not affected except at very low rainfall acidities (pH 2.7 and 2.3) (Evans et al, 1981b).

Irving and Miller (1977,78) reported higher photosynthetic rates, and lower diffusive resistances in field-grown soybeans exposed to simulated acidic rain. These experiments were conducted at Argonne, in the United States Midwest, where ambient pH levels are not as low as in New York. Chlorophyll content was 29% higher ($P<0.1$) in plants receiving the acid simulant (pH 3.0) than in control plants at the end of the growing season. However, no statistically significant changes in soybean seed yield were observed due to the application of acidic rain.

The apparent lack of agreement in the results of these field studies examining the effects of simulated acid rain on soybeans are not surprising if one compares the conditions under which the various experiments took place. These experiments differed in the soil type that was used, the climatic conditions, when the spraying took place, the number of rainfall events and the pH of ambient rainfalls received by the soybeans. Experiments with soybeans at Brookhaven, New York (Evans et al, 1980) used a very large sample size, with 30 plots for each treatment, and soybeans were sprayed 3 times weekly from June to September (41 rainfalls). In contrast, a much smaller sample size was used for soybean experiments at Argonne (Irving and Miller, 1977,78) and the simulated rain (pH 3.0) was applied only eight times during the growing season, beginning at the end of July (i.e. plants were older at the time spraying began than in Evans' experiments). Differences in the age of plants at the time when they were exposed to acidic precipitation, and in the number of rainfall exposures, as well as a smaller sample size, could account for the lack of effect of acidic precipitation on soybean yield in the Argonne study.

Experiments have shown that factors such as the atmospheric concentration of gaseous air pollutants, and the nutrition of plants may alter the response of plants to acidic precipitation. Jacobson et al (1980) investigated the impact of acidic rain on the growth of field-grown soybeans in combination with treatments of low and high concentrations of ozone. When simulated acidic rain at pH levels of 2.8, 3.4, and 4.0 was applied to field-grown soybeans at low ozone levels (average hourly concentration did not exceed 0.03 ppm), more acidic treatments produced a shift in partitioning of photosynthate from vegetative (stems and leaves) to reproductive organs (seeds). No shift in photosynthate partitioning was observed when simulated acidic rain was applied to soybeans exposed to ozone concentrations that reached a maximum of 0.125 ppm, and significantly reduced growth and yield. The depression of growth and yield was greatest with the most acidic rain treatment. Simulated acidic rain and multiple exposures to ozone has also been reported to decrease the foliar dry weight of red kidney beans in laboratory tests (Shriner, 1978b). The manner in which acidic precipitation may interact with gaseous pollutants in causing effects on vegetation has not received adequate attention in the scientific literature.

The effects of acidic precipitation on growth and yield of vegetation may be altered by differences in the nitrate and sulphate concentrations in the rainfall. Experiments have suggested that positive growth responses to nitrogen and sulphur in simulated rain treatments may occur for some species. Jacobson et al (1980) obtained the best growth of lettuce by treatment with simulated acidic rain at pH 3.2 containing high sulphate:nitrate ratios. These results suggest that sulphur may have been limiting in the nutrition of these plants. Low levels of SO_2 have previously been reported to increase yield through correction of sulphur deficiency in ryegrass (Cowling et al, 1973). Wood and Bormann (1974), Lee and Weber (1979) and Tveite and Abrahamsen (1980) have reported evidence from experiments with simulated acidic rain treatments, suggesting a positive growth response due to nitrogen and sulphur in tree species. Wood and Bormann cautioned however, that the increased productivity of pine seedlings noted at pH 2.3 may have been a short-term phenomenon, since large decreases in exchangeable K, Mg and Ca of the soil were brought about by leaching from acidic rainfalls (pH 4.0 to 2.3). The changes that occur to soils from acid inputs (e.g. effects on soil nutrient status, increased Al and Mn concentrations, slower rates of nitrification) that may indirectly affect plant growth will not be discussed here. Several excellent reviews consider the effects of acidic precipitation on soils and their potential implications to plant growth (Wiklander, 1974,79; Overrein, 1978; Overrein et al, 1980; McFee, 1980). The general concensus of most scientists working in this area (1978 Nato conference on effects of acid precipitation on terrestrial ecosystems, eds. Hutchinson and Havas, 1980) is that any effects of acidic rain on agricultural soils are very unlikely because these soils are intensively managed (fertilizer and lime additions would maintain the level of nutrients and the soil pH), but that certain forest soils (soils that are poorly buffered, i.e., have low cation exchange capacity due to low clay and organic matter contents) may be susceptible to changes from increased H ion inputs in precipitation.

At present there is no conclusive evidence to support the supposition of declining forest productivity due to acid rain. Jonsson and Sundberg (1972) compared growth data (based on tree-ring analyses of past diameter growth) from two regions in Sweden differing in susceptibility to acidification. They found reductions in forest productivity of 0.3 - 0.6% in the susceptible region and they attributed this reduction to acidification. Although many naturally occurring variables were considered in this attempt to detect growth reduction, it seems doubtful that such small changes in productivity could be detected among much larger natural fluctuations in productivity that exist due to annual climatic variations. Similar approaches used by Abrahamsen et al (1976) and Cogbill (1975), in Scandanavia and North America, have not succeeded in correlating acidic precipitation occurrences to forest growth. Tamm et al (1977) were also unable to detect negative effects on growth of pine forests as a result of additions of H_2SO_4 over an eight year period (1969 - 1977). However, even moderate additions of H_2SO_4 or S to these forest plots had a significant effect on soil biological processes, in particular in turn-over rates for nitrogen. These changes in soil processes suggest that in the long term, soils and in turn, forest growth would be affected by acid rain.

Studies have shown that seedling establishment is one aspect of plant growth which is particularly sensitive to hydrogen ion concentration. Abrahamsen et al (1975) and Teigen (1975) investigated the impact of simulated acid rain additions to a mineral soil on germination and seedling establishment of Norway spruce and Scots pine. In a soil pH range of 3.8 to 5.6 germination of these species had a broad optimum around pH 4.8. Seedling establishment showed a higher sensitivity to soil acidity. At pH values <4.2 a pronounced effect was observed on seedling establishment. About 80% of the seeds did not develop normal seedlings at pH 3.8. Lee and Weber (1979) exposed seeds of eleven woody species to 2.3 cm/week of simulated sulphuric acid rain at pH values of 3.0, 3.5 and 4.0, and to a simulated control rain of pH 5.6. Eight species were affected by acidic rain treatments, although the direction and magnitude of effects varied with species and with treatment. Seedling emergence was stimulated by at least one acid treatment for four species and inhibited for one species. Top growth was stimulated for at least one acid treatment for four species, while root growth was inhibited for one species. Inhibition of yellow birch seedling growth was observed by Wood and Bormann (1974) for simulated sulphuric acid mist at pH 2.3, but not at pH values of 3.0 or higher. These studies suggest that some acid rain events, at concentrations typical of Ontario and the northeastern United States, are sufficient to affect seedling establishment and growth of a large percentage of forest woody species.

Experimental results have also shown that pollen germination and pollen tube growth in forest species is inhibited by the concentration of H ions (pH's) present in ambient rainfall. Cox (1982) investigated the effect of acid precipitation on reproductive processes of some eastern Canadian forest flora species to simulated acidic rain, alone and in combination with cadmium. Pollen from all 13 species tested showed a significant response to a decrease in pH of the culture medium (pH 5.6 to 2.6). Both percent germination and pollen tube elongation were inhibited at lower pH's (3.6 to 2.6), whereas decreases from pH 5.6 to 4.0 showed both stimulatory and inhibitory responses of these processes, depending on the species. At low concentrations of Cd (i.e. those expected in ambient rainfall) stimulations of pollen germination and tube elongation occurred when compared to simulated rainfall with no Cd additions. Experimental results with bracken fern

(Pteridium aquilinum) have also shown significant inhibition of the motility of male gametes (spermatozoid) during exposure to buffered solutions at pH 4.5 (2%) when compared to the pH 6.1 treatment (65%) (Evans and Bozzone, 1977,78). In subsequent studies with bracken fern similar results were obtained under forest conditions (Evans and Conway, 1980). These studies suggest that the pH levels of ambient rainfall, may be inhibiting reproductive processes in forest tree and herbaceous species. However, the overall impact of this to these species is not known at present, since no estimates of the role of seedling establishment in the perpetuation of these species has been made nor is it clear what is the relation between pollen tube inhibition and reduction in seed set or viability. The recent studies by Cox (1982) of the effects of acidic precipitation on reproductive processes could explain the decrease in seed yield (i.e. less pods/plant and less seeds/pod) observed for some agricultural crops, such as pinto beans (Evans and Lewin, 1981). Examination of the effect of decreased pollen germination and pollen tube growth in crop species is necessary in order to determine their effects on vegetable and fruit production.

Leaching of nutrients from foliage

Leaching of nutrient cations from leaves of plants involves exchange reactions in which hydrogen ions in rainwater replace cations on exchange sites of the leaf cuticle (Tukey, 1970a). As a result of increased acidity in precipitation, alterations in the rate at which nutrients are leached from foliage may occur. Moreover, reduced levels of nutrients in plants may affect their productivity or the quality of produce.

Wood and Bormann (1975) reported that increased rainfall acidity led to increases in foliar losses of potassium, magnesium and calcium from bean and maple seedlings, while sodium levels were unaffected. At pH values of 3.0 and below, tissue damage and the resulting loss of cell contents to the leachate, was probably the primary factor resulting in large losses of nutrients. Above pH 3.0 no visible tissue damage was observed on the foliage, suggesting that increased leaching was the result of increased cation exchange with H^+ in the leaching solution. Horntvedt (1977, 78) in experiments with spruce similarly observed foliar leaching of calcium, potassium, and to a lesser extent, magnesium from tree crowns, with no visible injuries. Increasing the acidity of the leaching solution had a significant effect on the loss of magnesium from the two woodland mosses Dicranum scoparium and Hypnum cupressiforme (Fairfax and Lepp, 1976). In tobacco leaves, calcium leached faster from foliage exposed to simulated rain of pH 3.0 than from foliage exposed to pH 6.7, whereas the amount of potassium leached was greater from the leaves exposed to the pH 6.7 simulated rain than from leaves exposed to pH 3.0. Little change occurred in levels of magnesium lost (Fairfax and Lepp, 1975). Evans et al (1981a) have also reported that more potassium was leached from foliage of pinto beans exposed to a control rainfall (pH 5.6) than from foliage treated with simulated rain at pH levels of 2.7, 2.9 and 3.1. Greater amounts of calcium, nitrate and sulphate were leached from the pinto beans by the acidic rainfall, while the amounts of ammonium, magnesium and zinc were the same at all of the pH levels. Reductions in foliar concentrations of nitrogen, calcium, phosphorous, and magnesium occurred when bush beans were exposed to an acidic mist, whereas potassium concentrations were not affected (Hindawi et al, 1977).

At the present time, insufficient data exist to evaluate the effect of nutrient leaching on productivity of crops and forests. Under field conditions the cation content of throughfall represents not only those cations leached from the leaves; large amounts may also be deposited in the canopy by dry fallout and washed out by rain. In a detailed study of the throughfall and stemflow chemistry of a northern hardwood forest at Hubbard Brook it was estimated that at least 27% of the leaching which occurred from the forest canopy could be accounted for by hydrogen ion exchange from the rain (Eaton _et al_, 1973). The mean pH of the precipitation at Hubbard Brook, during this study was 4.06, suggesting that significant alterations to the cycling of nutrients in forest ecosystems in large tracts of land in the northeastern United States and in eastern Canada are occurring at the present time.

While research on the effect of acidic precipitation on leaching of mineral nutrients from foliage has received considerable attention in recent years, there have been no studies of changes that may occur in the leaching rate of other important foliar substances such as amino acids, carbohydrates and growth regulators.

Effects of acidic precipitation on microbial parasites of terrestrial plants

Very little is known about the possible effects of acidic precipitation on relationships between host plants and their microbial pathogens. Many microorganisms inhabit the surfaces of higher plants and these may have either a pathogenic (negative), beneficial, or neutral influence upon the growth, development and reproductive capability of the host plant (see reviews: Preece and Dickinson, 1971;

Dickinson and Preece, 1976). Since many bacteria are inhibited in acidic environments, this suggests that bacterial plant pathogens and symbionts may be susceptible to acidic precipitation. Shriner (1978a) reported on the effects of acidic precipitation on various host-parasite interactions. Simulated acidic rain (pH 3.2) resulted in: (1) an 86% inhibition in the number of telia of Cronartium fusiform on willow oak (Quercus phellos); (2) a 66% inhibition in the reproduction of root-knot nematode (Meloidogyne halpa) on field-grown kidney beans (Phaseolus vulgaris); and (3) a 29% reduction in the percentage leaf area of field-grown kidney beans affected by Uromyces phaseoli. The development of halo blight on kidney bean (caused by Pseudomonas phaseolicola) was either stimulated or inhibited by acidic rain, depending upon the stage in the disease cycle when the rainfalls occurred. Simulated acidic rain inhibited initial infection but stimulated development of the pathogen after infection. Another study involving the relationship between host and microorganism (Shriner and Johnston, 1981) looked at the effect of simulated acidified rain on nodulation of greenhouse- and field-grown kidney beans (Phaseolus vulgaris) and greenhouse-grown soybeans (Glycine max) by Rhizobium spp.. Nodulation of both kidney beans and soybeans exposed to acidic rain (pH 3.2) was inhibited by 73%. Neither nitrogenase activity per plant nor nitrogenase activity per nodule were consistently inhibited, however. For kidney beans, inhibition of nodule formation occurred when "rain" of pH 3.2 was applied only to the foliage, as well as when applied only to the soil, or to both the soil and foliage. In in vitro tests, germination and growth of Alternaria solani, a pathogen of tomato leaves, was inhibited under conditions more acid than pH 5.0

(Leben, 1954). Reduced nitrogen fixation has also been reported for epiphytic lichens exposed to simulated acid rain of pH 4.0 and below (Denison *et al*, 1977).

Effects of acidic precipitation on diffusion resistance of foliage

Evans *et al* (1981a) measured leaf (stomatal) resistance in *Phaseolus vulgaris* exposed to rain of pH 5.6, 3.4, 3.1 and 2.7. Exposure to simulated acid rain of pH 2.7 to 3.4 (no visible injury at pH 3.1 and 3.4) decreased diffusion resistance significantly. These results suggest that acidic precipitation may affect the ability of guard cells to function normally. This alteration in diffusive resistance may influence other important functions such as photosynthesis, and resistance to drought stress, pathogens and gaseous air pollutants.

Features of plants that confer tolerance to acidity of precipitation

It has been known for some time that plants differ greatly in their sensitivity to SO_2 and other toxic substances (Linzon, 1972). From the present review of the literature on the susceptibility of plants to acidic rain, it is clear that species also differ greatly in their sensitivity to this pollutant. One question to be asked then is "What characteristics of a plant determine its sensitivity?". Research has been singularly lacking in an attempt to identify common responses amongst species to simulated acid rain which might influence their sensitivity.

Shriner (1981) has suggested that the sensitivity of foliage to injury may be related to its wettability. There are several factors that determine the degree to which leaf surfaces become wet, and hence the severity of foliar injury. Leaves which have a thick, waxy cuticle resist the penetration of aqueous solutions and protect sensitive leaf tissues. Wettability of leaf surfaces increases markedly when the cuticle, and thus the epicuticular waxes, are removed. The main pathway of entry of solutions into leaves is thought to be through micropores in the cuticle (Schönherr and Bukovac, 1970; McFarlane, 1974). Penetration of rain through stomata is probably insignificant, if it occurs at all (Greene and Bukovac, 1974). Cuticular pores may be more numerous at the base of certain specialized epidermal cells, such as trichomes, hydathodes or glandular hairs, allowing solutions to penetrate faster at these locations. This could explain why injury from acidic precipitation occurs more frequently at these sites (Evans et al, 1977b). Leaf orientation and morphology are also important in determining leaf wettability. Leaves which are oriented vertically will shed water, while those which are held in a horizontal plane will retain rain droplets for a longer period of time. As these droplets evaporate on the leaf surface, the acidity increases due to the concentration of hydrogen ions. In some leaves the vascular tissue is sunken, forming troughs in which solutions can pool and increasing the risk of injury at these sites (Evans et al, 1978; Evans and Curry, 1979; Evans, 1980).

Experimental studies by Evans et al (1978) and Evans and Curry (1979), showed substantial differences in plant response and these were attributed, at least partially, to histological responses to acidic rain. Species in which hypertrophy (abnormal cell enlargement) or hyperplasia (abnormal cell division) were observed to occur showed a greater resistance to foliar injury. These responses may function by reducing the amount of intercellular space and thereby reducing the movement of acid solutions. This mechanism needs to be examined in more detail in a greater number of species.

Summary of plant features which are important in sensitivity to acid rain

Table 4 summarizes a number of the plant processes and characteristics that may be critical to the sensitivity of plants to injury from the acidity of precipitation (Jacobson, 1980).

Table 4. Plant processes and characteristics that confer tolerance to acidity of precipitation (after Jacobson, 1980).

Exclusion
Leaf surface features (trichomes, stomates, vascular bundles, cuticle thickness)
Leaf orientation and morphology
Chemical composition of cuticle
Histological responses of leaves (hypertrophy and hyperplasia)
Flower orientation
Protection of sexual organs
Pollination mechanism

Neutralization
Salts on leaf surfaces
Buffering capacity of leaves

Metabolic Feedback Reactions
Enzymatic reactions that consume hydrogen ions or yield alkaline products

Curiously, most of these ideas have not been tested in experimental studies, thus definitive examination of these theories is lacking. Knowledge of which characteristics of a plant impart resistance is extremely important since it could then be used to predict tree, crop and understory species which are especially at risk and could be used in crop breeding.

THE REASEARCH PROJECT

Reasons for using Artemisia tilesii in the present study

The genus Artemisia has a widespread distribution throughout the world, with many of the species known for their ability to accumulate high levels of salts in their tissues (Waisel, 1972; Chapman, 1975). High concentrations of such salts on or at the leaf surface could permit neutralization of acidic droplets on the leaf surface.

Artemisia tilesii, the particular species of interest in this study, was found growing in the Smoking Hills area of the Northwest Territories where intense natural fumigation of SO_2 and acid aerosol occur as a result of the spontaneous combustion of bituminous shales in arctic sea-cliffs. Artemisia tilesii plants in the Smoking Hills population are remarkably tolerant to the field conditions and rarely show SO_2 damage even under exposures of 0.5 - 1.0 ppm SO_2 which last for several days (Hutchinson et al, 1978). Acidic droplets which form on the leaf surface as a result of the dissolution of dry-deposited sulphate under conditions of dew or fog (which occur frequently), or the occasional rainfall, are an additional stress.

An interesting observation made by Dr. Hutchinson at the Smoking Hills was that A. tilesii plants were able to neutralize very acidic droplets falling on the leaf surface to a $pH<5.0$ within 30 minutes of the rain ceasing, while other species less tolerant of the polluted conditions retained droplets of approximately pH 2.0 on their foliage. This observation suggested that the acid tolerance shown by A. tilesii might be related to the ability of its foliage to neutralize or buffer acidic droplets, possibly through translocation of cations onto the leaf surface. An internal buffering of pH may equally cope with SO_2

injury in A. tilesii or other species.

Individual clones in the Smoking Hills population were very variable in their morphology (leaf orientation, leaf size and shape, leaf dissection, plant stature etc.). This species, therefore, provided a unique opportunity to test some ideas on tolerance to acidic rain associated with morphological features.

Research objectives

The objectives of this thesis, which involve an experimental study of the effects of simulated acidic rain on foliage and on growth of *Artemisia tilesii* under greenhouse conditions, are:

1. to describe the effects of simulated acidic rain on foliar injury, growth and foliar leaching of *A. tilesii* clones from the Smoking Hills and from a control (unpolluted) population.
2. to investigate whether morphological features of *A. tilesii* clones are related to the amount of injury sustained from simulated acid rain.
3. to describe the histological responses of foliage of *A. tilesii* clones to acidic rain, in view of the hypothesis that hypertrophy (abnormal cell enlargement) and hyperplasia (abnormal cell division) are responses that occur in plants which are acid rain resistant (Evans *et al*, 1978; Evans and Curry, 1979).
4. to examine leaf surface responses to acidic rain using scanning electron microscopy, and to test the hypothesis that leaf surface features such as hydathodes, trichomes, glandular hairs, stomates, or vascular bundles, are preferential sites of injury (Evans *et al*, 1977b, Evans, 1980).
5. to determine if "invisible" (microscopic) injury to the foliage occurs in the absence of "visible" (macroscopic) injury.
6. to test the hypothesis that buffering capacity of leaves may protect against injury from acidic rain, using *A. tilesii* and other species ranging in their sensitivity to simulated acidic rain.
7. to determine if the Smoking Hills population is more acid rain tolerant than *A. tilesii* from a control site, as a result of its selection in an acid-stressed environment.
8. to compare the tolerance of *A. tilesii* to simulated acid rain with that of other species.

MATERIALS AND METHODS

Description of plant material, growth conditions and rainfall application procedure

A. Plant material used

Clonal plants of Artemisia tilesii were obtained from the Smoking Hills, N.W.T. and from Alaska courtesy of Dr. Jay McKendrick. The plants at the Smoking Hills location were exposed to high concentrations of SO_2 and acid aerosol which occur as a result of the spontaneous combustion of bituminous shales in these arctic sea-cliffs (Hutchinson et al, 1978). A complete description of this unique site, including details of the physical and chemical characteristics of the soil and atmosphere is given in Gizyn (1980) and Havas (1980). Artemisia tilesii at the Smoking Hills grows in close proximity (80 m) to the SO_2 source, in a zone in which most other species have been eliminated (Gizyn, 1980). Even under exposures of 0.5 - 1.0 ppm SO_2 continuously for several days, damage to A. tilesii plants is seldom apparent, indicating remarkable tolerance (Hutchinson et al, 1978). The clones provided by Dr. McKendrick were obtained from a Palmer, Alaskan population (Control) which was well removed from any significant source of SO_2.

Phaseolus vulgaris L. "7 pencil pod wax" (wax bean) and Spinacea oleracea L. "Longstanding bloomsdale" (spinach) were used in comparative studies and were grown from seed. Phaseolus vulgaris was selected for comparison with Artemisia tilesii because it had been used extensively in experiments with simulated acid rain (Ferenbaugh, 1974; Wood and Bormann, 1975; Evans et al, 1977a,b; Jacobson and Leuken, 1977). Although Spinacea oleracea has been used less extensively in simulated acid rain studies (Jacobson and Leuken, 1977; Lee et al, 1981) its foliage appeared to be highly sensitive to acidified rain solutions. In our own experiments in 1981 spinach also showed acid lesions on foliage treated with simulated rain at pH 3.0 (Hutchinson and Adams, in preparation).

B. General growth conditions

Clones of Artemisia tilesii were grown in the Department of Botany greenhouses, in 46 x 30 cm wooden flats, for at least four months prior to experiments. The flats were filled with a basic soil mixture (pH 7.9) containing equal parts of loam, peat and sand. These "stock" plants were watered every day in the morning and fertilized on alternate weeks with 20-20-20 (Plant-Prod).

Stem cuttings of six clones from the Smoking Hills and three Alaskan clones were used in rain simulation experiments as follows:

Smoking Hills (SH) Clone no. 3 5 8 9 11 12
Alaska (AL) Clone no. 2 3 5

(Material was quite limited in availability and this was recognized as a limitation in inter-population comparisons). Basal ends of 2.5 cm stem cuttings were dipped in root hormone (Stim-root 1, 0.1% indole-3butyric acid) and allowed to root in perlite for two weeks. Plants were subsequently transplanted into 7.6 cm diameter plastic pots, filled with pasteurized soil of the above mixture, and allowed to establish for 3 weeks to ensure adequate development of a root system before acid rain treatments.

Phaseolus vulgaris and *Spinacea oleracea* seeds were planted in 10 cm diameter plastic pots filled with the standard greenhouse potting mix. They were allowed to grow for 22 and 30 days respectively under fluorescent lights at about 20°C in the greenhouse prior to acid rain treatments.

Since experiments with *Artemisia tilesii* continued throughout the year, these plants were grown under a wide range of light and temperature regimes in the greenhouse. Accordingly, during the winter and spring months plants were kept at about 20°C, and natural light was supplemented with fluorescent light, thus extending the photoperiod to 14 hours. All of the bean and spinach plants were grown under these conditions. During the summer *Artemisia* plants received only natural sunlight and blinds on the greenhouse roof were lowered to moderate temperatures.

Plants were fitted with plastic coffee cup lids (Canada Cup 250) during rainfall applications to minimize entry of solutions to the soil. The interest was on foliar effects of acid rain rather than indirect effects on growth via N or S inputs to soil. During the experiments the plants were bottom watered with tap water by filling petri dishes under each pot. No change was observed in soil pH during the experiments (Table 45).

C. Rainfall solutions and application procedures

Rainfall solutions with pH values of 5.6, 4.0, 3.5, 3.0, 2.5, and 2.0 were used in rain simulation experiments. Table 5 gives the concentration of hydrogen ions present in simulated rain solutions at each pH level.

Table 5. Hydrogen ion concentration of the simulated rain solutions at various pH levels.

pH	Hydrogen ion concentration (μeql^{-1})* [H_3O^+]
5.6	2.5
4.0	100
3.5	316
3.0	1000
2.5	3160
2.0	10000

*Derived from the equation $pH = -\log_{10}[H^+]$

Rainfall uncontaminated by dust and by strong acid radicals has a pH of approximately 5.6, due to the dissolution of CO_2 from the atmosphere. The 4.0 pH value is representative of average values currently found in most of eastern North America and even some parts of western North America (NADP DATA REPORT, 1980). pH values between 3.0 - 3.5 are the lower limit of the acidity recorded for rainfall, nevertheless these low values occur several times in a season in Ontario (Table 3) and the northeastern United States (Ministry of the Environment, unpublished; NADP DATA REPORT, 1980). The pH 2.0 and 2.5 treatments therefore represent either extreme and rare events (2.5) or extreme and as yet unrecorded events (2.0). Isolated storms of pH 2.1 have been reported (Likens and Bormann, 1974). Based on the amounts and presumed stoichiometric balance among ions in natural rain, sulphuric acid probably accounts for about 2/3 of the total acidity currently observed in precipitation in eastern North America (Cogbill and Likens, 1974; Ministry of the Environment, unpublished). The relative contribution of sulphuric acid to the total acidity at the Smoking Hills is much greater since the source is entirely SO_2 (Hutchinson _et al_, 1978). Since sulphuric acid was shown to contribute most to the acidity of precipitation, rain solutions for initial experiments were prepared by dilution of "Baker analyzed" reagent grade sulphuric acid with distilled water.

To check for possible differences in effects related to other constituents of natural rainwater, a simulated solution containing concentrations of anions and cations present in rain, was used in latter experiments. This rain solution ("B") was prepared according to the simulated rain recipe used by Evans (personal communication) at Brookhaven National Laboratory, and based on analyses of summer rainfall in the northeastern United States (Table 6).

Table 6. Constituents of simulated rain solution ("B")(Evans, personal communication).

Compound	Molecular weight	µg/1
$(NH_4)_2SO_4$	132.14	190.28
NH_4Cl	53.50	1140.30
$NaNO_3$ anhydrous	85.01	85.86
NaCl anhydrous	58.45	1650.04
NaF	42.00	220.92
$CaCl_2$	110.99	320.76
$MgSO_4.7H_2O$	246.49	788.77
KCl	74.55	276.58
$Zn(NO_3)_2.6H_2O$	297.49	592.01
$Ni(NO_3)_2\ 6H_2O$	290.80	247.18
$CuSO_4.5H_2O$	249.68	746.54
$Cd(NO_3)_2.4H_2O$	308.49	109.51
$MnSO_4.1H_2O$	169.01	92.11
$Pb(NO_3)_2$	331.20	2045.16

A concentrated stock solution (to make 1000 litres of rain solution) of this mix was used, with the NaF and $Pb(NO_3)_2$ excluded, so as to avoid formation of a precipitate in the concentrated solution. Rain solutions were then made up in 50 litre batches by mixing the NaF, $Pb(NO_3)_2$ and 50 ml of stock solution in enough distilled water to bring the total volume to 50 litres. The acidity of the rain was adjusted by adding Baker "analyzed" reagent grade sulphuric acid and analytical grade nitric acid in a sulphate to nitrate molar ratio of 2.5:1. This ratio is based on analyses of summer rainfall events at the Brookhaven National Laboratory in New York (Raynor and Hayes, 1978) and in Ontario (Ministry of the Environment, personal communication). Data for concentrations of anions and cations in Ontario rainfall were not available at the outset of this study. However, differences in the constituents of the rain were not expected to be large between Ontario and northeastern United States rainfall.

All rainfall solutions were adjusted to the desired pH using an Orion Research Model 801 digital pH meter, calibrated using certified buffer solutions. The accuracy of these rainfall solutions was controlled to within ± .002 units of pH 2.0, 2.5, 3.0, 3.5 and 4.0 and within ± .01 units of pH 5.6.

A spraying apparatus, consisting of 5 exposure chambers, was developed and positioned under a bank of fluorescent lights in the greenhouse to spray controlled amounts of artificial rain onto plants positioned on a greenhouse bench. Each chamber was equipped with two atomizing nozzles (PaTe Nozzles) which distributed "rain" over an area 0.91 m x 1.45 m in a fairly uniform manner. Since plants were placed into the chambers randomly each time they were sprayed, and never in the same position, small differences in the volume of rainfall received within the chamber were considered to be insignificant. A schematic diagram of the spraying apparatus is shown in Figure 1. The chambers were separated by plastic dividers on two sides and at the ends plastic curtains were fashioned. These could be opened to move plants in and out of the chambers. pH treatments were assigned randomly to the 5 exposure chambers. A pump (Little Giant Pump Co., Model 532001 35-0M) was used to move rainfall solutions from 20 litre polypropylene carboys, in which they were stored, through ordinary plastic garden hosing into the atomizing nozzles. The whole system was thoroughly rinsed with tap water and a small amount of "rain" of the pH to be used in the subsequent exposure, between exposures of different acidity. Plants were exposed to treatments in order of increasing acidity.

The diameter of rain droplets produced by the spray nozzles and the intensity of the "rainfall" were measured for comparison with natural rain (Table 7). The simulation system had a droplet size (0.20 - 0.40 mm) comparable to those reported for light to moderate showers (Shriner _et al_, 1977), but produced rainfall at a rate greater than that associated with "moderate intensity" natural rains. For measurements of droplet size, microscope slides, which had been smeared with vaseline and then warmed over a bunsen burner to obtain a smooth coating, were held at plant height in the chambers for a few seconds during spraying, covered with a petri dish and then removed. Droplet diameters were determined with a microscope and a calibrated occular micrometer and the mean diameter calculated. Rainfall intensity was determined by using rain gauges placed at a number of positions in the exposure chambers. Based on the data for rainfall intensity, 2 exposures of 20 minutes each week gave a total deposition of 2.0 cm.

Figure 1 Schematic representation of the misting apparatus designed to simulate acid rainfall.

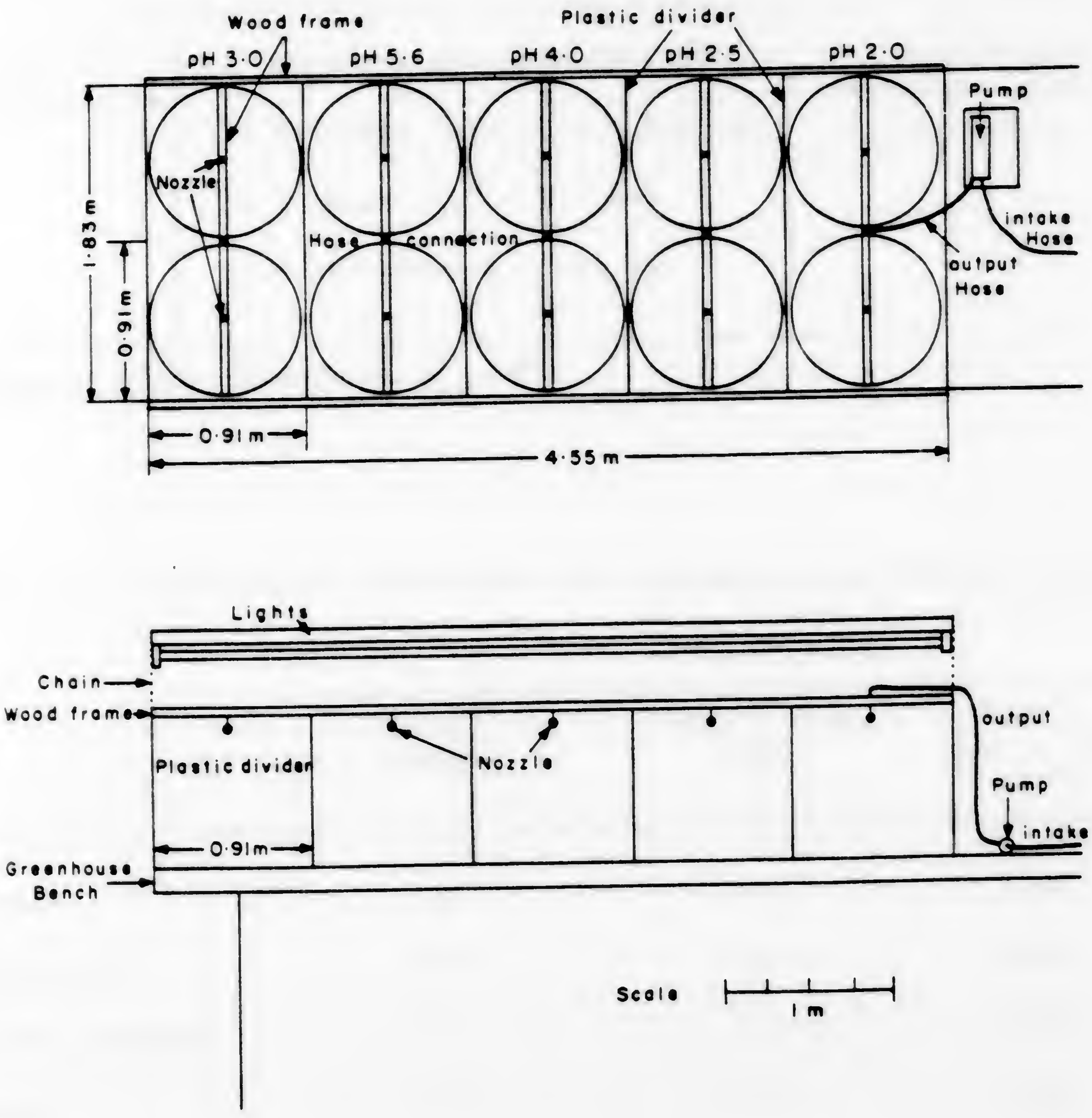


Table 7. Comparison of droplet size and rainfall intensity of simulated precipitation and of natural precipitation.

	Droplet diameter (mm)	Rainfall intensity (cm/hr)
Simulated precipitation	0.20 - 0.40 (range)	3.0
Natural precipitation	1) Steady light rain (0.1 mm) 2) Moderate rain (0.1 - 1.0 mm) 3) Showers (1.0 - 2.5 mm) 4) Heavy showers (3.05 mm)	1.27 - 2.54 (moderate intensity)

Source of Data: Shriner *et al*, 1977.

Table 8. Precipitation statistics for representative Ontario stations.

	Precipitation		
	Annual (cm)	Apr. - Sept. (cm)	Weekly Apr. - Sept. (cm)
Ottawa	85	45	1.88
Brockville	97	48	2.00
Toronto Airport	75	41	1.71
London	92	47	1.96
Windsor	84	47	1.96
Sudbury	84	48	2.00
Kapuskasing	87	50	2.08

Source of Data: Hare, F. K. and Thomas, M. K., Climate Canada. Toronto: Wiley, 1974.

This amount of rainfall is close to the average of 1.94 cm/week for 7 stations in Ontario for the months April to September, i.e. the growing season (Table 8). The term "rain" will be used throughout this thesis to refer to the acidified rainfall solutions applied to the plants with this apparatus.

D. Conditions for individual rain simulation experiments

1. Effects on Smoking Hills clones of *Artemisia tilesii* (SH 3, SH 5, SH 8, SH 9, SH 11, and SH 12).

For each of the six clones 50 ramets were divided into 5 groups. Sulphuric acid solutions with pH values of 5.6, 4.0, 3.0, 2.5, and 2.0 were applied 3 days a week for 6 minutes. A total of 12 rainfalls was applied over 4 weeks. Total weekly deposition was 0.9 cm. A fully randomized pot placement was used within each of the exposure chambers. Overhead fluorescent lights supplimented natural light for 14 hours daily.

2. Dose-response study

Plants of clones 5 and 8 from the Smoking Hills were used to investigate the effects of spray frequency and duration. These two clones had shown similar sensitivity to leaf injury in a previous study. Half of the 144 plants were sprayed every day for 6 days while the remainder were sprayed on alternate days for 12 days i.e. total dosage was the same. Sulphuric acid solutions of pH 5.6, 3.0 and 2.0 were applied to the plants for 2.5, 5, 10 or 20 minutes. The total rainfall deposition was 0.75 cm, 1.5 cm, 3.0 cm and 6.0 cm respectively, over either 1 or 2 weeks, depending on the frequency of the applications. A fully randomized block design was used. Plants were moved into the exposure chambers randomly and placed back in their randomized blocks only when all foliage was dry. Overhead fluorescent lights supplemented natural light for fourteen hours daily.

3. Effects on *Artemisia tilesii* from an acidic (Smoking Hills) and a control (Alaska) site

For each of six clones (SH8, SH9, SH11, AL2, AL3, AL 5) 70 ramets were divided into 5 groups. Sulphuric acid solutions with pH values of 5.6, 4.0, 3.5, 3.0 and 2.5 were applied 2 days a week for 20 minutes. Total weekly deposition was 2.0 cm. At the end of 6 weeks (12 rainfalls) 35 plants from the control and 35 plants from each of the treatments were harvested for each clone for dry weight determination. The remainder of the plants received rainfall applications for an additional 6 weeks (total 24 rainfalls). This experiment was continued for the 12 weeks in order to simulate the effect of continuous exposure to acid rain over a growing season. Most previous work has been concerned with short term exposures and acute effects rather than with chronic effects. A fully randomized block design was used in this experiment. Plants were moved into the exposure chambers randomly and placed back in the randomized blocks when the foliage was dry as before. The only light received by the plants was natural sunlight.

4. Comparison of the response of two other herbaceous species with *Artemisia tilesii*

Fifty plants each of *Artemisia tilesii* (two populations), *Phaseolus vulgaris* and *Spinacea oleracea* were treated with a simulated rain solution ("B"), of pH 5.6, 4.0, 3.5, 3.0 and 2.5, 2 days a week for 20 minutes. Plants were exposed to rainfall 4 times over a 2 week period. Total weekly deposition was 2.0 cm. A fully randomized block design was used. Overhead fluorescent lights supplemented natural light for 14 hours daily.

Foliar injury

A. Determination of macroscopic injury

Foliage of acid-treated plants was examined at regular intervals for the presence of any acid rain injury. Scoring was carried out 24 hours after rainfall was applied. This was found to allow sufficient time for damage to develop. If noticeable injury was present, control plants were examined for the same symptoms. Only when these symptoms were absent from control plants were these characteristics attributed to acid rain sprays. On one occasion damage from two-spotted spider mites occurred on control and acid-sprayed foliage.

Plants were scored according to the number of leaves out of the total present which showed foliar damage ascribable to acid sprays. For selected leaves, of the same age (fully-expanded), the fraction of leaf area showing acid rain injury was also estimated, according to a ranking system. The system that was used to do this was a modified Horsfall-Barratt (Horsfall and Barratt, 1945) ranking scale which allowed more precise determination of foliar injury at the extremes.

This scale accounts for the fact that greater accuracy in visual estimation of leaf damage is possible at the two extreme ends of the scale than in the middle. An average value for percent leaf area injured was then calculated using the mid-values of each class.

Table 9. Modified Horsfall-Barratt ranking scale that was used to estimate the percentage of leaf area injured.

Rank	Percent leaf area injured (%)
1	0
2	0-5
3	5-10
4	10-25
5	25-50
6	50-75
7	75-90
8	90-95
9	95-99
10	100

B. Determination of microscopic injury

1. Leaf clearing preparation

Leaf tissue of *A. tilesii* only was cleared using an adaptation of the method outlined in Berlyn and Miksche (1976). Tissue taken from the same mid-region of leaves was also used in histological and SEM preparations. A minimum of 5 leaf samples were cleared for each pH treatment. Small samples (< 10.0 mm), taken from leaves of the same developmental age, were placed in hot 85% ethyl alcohol until most of the chlorophyll was removed. The samples were then placed in 5% NaOH, vacuum infiltrated, and left in capped vials until the tissue reached a light straw colour (approx. 8 hours). For some of the leaves, clearing was completed by transferring the specimens to a saturated chloral hydrate solution (250 gm of chloral hydrate per 100 ml distilled H_2O) and leaving for 2 days at 20°C. It was found however, that by placing tissue in a solution of 1% sodium hypochlorite (bleach) for approximately 2 minutes, this step could be eliminated and clearing was more satisfactory. After clearing was complete, specimens were washed gently in 6 changes of distilled water at 15 minute intervals and then dehydrated in a graded series of ethanol solutions. Subsequent to dehydration, specimens were stained with a 0.5% solution of safranin in 1:1 absolute EtOH-xylene overnight at 20°C. Staining was differentiated by passing the tissue through 5 - 6 changes of 100% EtOH, followed by 100% EtOH-xylene (1:1). Tissue was stored in xylene in capped vials and later mounted in Permount on a slide and covered with a cover slip. During the week following, additional Permount was required under the cover slip as drying progressed.

2. Histological preparation

A. tilesii plants, Smoking Hills clone 11 and Alaska clone 3, were used for histological observations made with the light microscope on plastic-embedded material. The method of sampling was to cut small pieces of leaf (approximately 1.5 mm X 1.5 mm) from an area in the center of fully expanded leaves of comparable age. Samples taken from acid-treated plants were selectively chosen to include a lesion, (diameter <1.5 mm), if one was present in this region of the leaf.

For fixation, leaves were covered in a cold 2% glutaraldehyde solution in 0.05 M phosphate buffer (pH 6.8), and tissue pieces removed with a razor blade. This tissue was then transferred to vials containing the same fixative. This primary fixation proceeded for 6 hours at 4°C. Following two 15 minute washes in the 0.05M phosphate buffer (pH 6.8), the samples were left overnight at 4°C in this same buffer. A final change of the phosphate buffer was made the next morning. The specimens were post-fixed in 1% osmium tetraoxide in

0.05 M phosphate, pH 6.8, for 3 hours at 4°C. After post-fixation the samples were washed with deionized-distilled water 3 times at 5 minute intervals. Dehydration was accomplished in a graded acetone series at 20°C. Each change was made 20 minutes apart and 5 changes of 100% acetone ensured complete dehydration of tissue. The tissue was embedded in Spurr's embedding medium (Spurr, 1969). Specimens were first placed in vials filled with a 2:1 mixture of acetone:Spurr (without the cure accelerator) and left uncapped overnight in a desiccator to evaporate the acetone. An equal amount of fresh Spurr medium (without the cure accelerator) was added to the vials each day for 2 days and mixed thoroughly. Specimens were placed in pure Spurr resin for 3 hours and then transferred to molds containing fresh embedding medium. Molds were placed in a 70°C oven overnight for the Spurr resin to cure. Blocks were sectioned with glass knives at 4 µm on a Porter-Blum ultramicrotome MT-2. Sections were placed in droplets of distilled water (5 sections per drop) on a glass slide which had been coated with a gelatin adhesive (Jensen, 1962). The slides were then placed on a hot-plate at 40°C to affix the sections to the slide. The sections were stained with Paragon Stain (Martin _et al_, 1966) prepared by dissolving 0.54 gm basic fuchsin and 1.46 gm toluidine blue in 30% ethanol to make 200 ml. Slides were placed in the staining solution at 60°C for 20 minutes, rinsed 4 times in distilled water, and dried on a hot-plate at 40°C.

3. Quantitative and qualitative assessment of cellular injury from leaf clearings and cross sections

Light microscopic measurements were made on cleared leaves to determine lesion diameter and frequency. Lesion diameter was determined using an occular micrometer that had been calibrated with a stage micrometer. The area of the field of view was also calibrated with a stage micrometer and used in calculations of lesion frequency. For each of six Smoking Hills clones four randomly selected fields were examined on each of five cleared leaves. These leaves were cleared after 12 six minute rain exposures of pH 5.6, 4.0, 3.0, 2.5 and 2.0. For two clones, SH 11 and Al 3, cleared leaves were examined after 1, 2, 4 and 8 - 20 minute exposures at pH 5.6, 3.5, 3.0 and 2.5, in collaboration with histological preparations and scanning electron microscopy. For these clones, a minimum of 10 randomly selected fields were examined for 4 replicate leaves. Measurements of lesions were categorized into four groups dependent on the diameter of the injured area (<250 µm, 250 - 1000 µm, 1000 - 2000 µm and >2000 µm). In most instances, lesions >1000 µm represented the maximum length of the damaged tissue, rather than its diameter since these lesions tended to be elongate in shape. For the majority of lesions however, the damaged area was circular in shape, forming under the droplets on the leaf surface. Leaf area injured was calculated from the measurements of lesion diameter using the formula πr^2.

Cell dimensions were measured from cross sections of plastic-embedded leaves using a Zeiss Jena Ergaval microscope fitted with a calibrated occular micrometer. Four leaves sectioned after 1, 2, 4 and 8 - 20 minute exposures at pH 5.6, 3.0 and 2.5 were examined for one Smoking Hills (SH 11) and one Alaskan (AL 3) clone. Height and diameter of 10 cells was measured for the upper epidermis, palisade mesophyll, upper and lower spongy mesophyll, and lower epidermis. In some acid-treated plants, cellular damage affected fewer than 10 cells and the sample size was thus reduced. Cell measurements of damaged tissue were standardized by measuring the most severely damaged tissue, determined by scanning all of the serial sections through a lesion. The following parameters were also recorded from cross sections: leaf thickness, lesion width and depth, total number of cell layers, number of cell layers damaged, locality of lesions with respect to vascular bundles, distance between vascular bundles and the width of vascular bundles. Qualitative assessments of lesion development were made by scoring for the presence of a number of injury symptoms (Table 10) ascribed to acid injury. This was done at the center of a lesion, as well as throughout the lesion to determine the differences in the severity of cellular damage across a lesion.

Table 10. Injury symptoms used in the qualitative assessment of lesion development.

Cell type	Injury symptoms noted
Upper epidermis	Staining affinity[a]; partial and complete collapse of cells
Palisade mesophyll	Staining affinity; chloroplast breakdown; cell hypertrophy or hyperplasia; cell collapse
Upper spongy mesophyll	Staining affinity; chloroplast breakdown; hypertrophy or hyperplasia; cell collapse
Lower spongy mesophyll	Staining affinity; cell hypertrophy or hyperplasia; cell collapse
Lower epidermis	Cell collapse
Vascular tissue	Damage to vascular bundle

[a] = Stained with Paragon Blue

Growth effects

A. Methods of harvesting and dry weight determinations

Plants of A. tilesii were removed from their pots and as much soil as possible removed from the roots, by gently shaking the soil away from the roots. The remaining soil adhering to the roots was removed by rinsing the roots gently in basins filled with water until they were clean. After drying the plants in an oven at 60°C for at least 24 hours the dry weight of the total plant and of the roots and shoots separately was determined. The shoot:root weight ratio was then calculated.

Initial fresh weights of 40 stem cuttings of Artemisia, 20 from the control and 20 for an acid treatment (pH 2.5), and final dry weights of these plants after four weeks of simulated rainfall, were also determined to establish whether there was a relationship between initial size and subsequent growth rate, under acid-stressed and control conditions. The results showed that within the range of initial weights (cuttings were all approximately one inch long) that there was no significant effect of size on the subsequent growth rate in either the control or acidic rain treatments.

B. Chlorophyll determination

The procedure used to determine chlorophyll was adapted from Arnon (1949). Leaves of similar age were taken from A. tilesii and ground in a mortar and pestle with a small quantity of acid-washed sand in 80% acetone. Samples of chlorophyll extract were made up to 10 ml with 80% acetone, centrifuged, and the optical density of the supernatant measured by a Pye Unicam 3P6-300 spectrophotometer at 645 nm and 665 nm. Appropriate dilutions were made when necessary using 80% acetone.

The concentrations of chlorophylls "a" and "b" and total chlorophyll were calculated according to the equations of Arnon (1949).

Chemical analyses

A. Microelectrode determination of droplet pH on leaf surfaces

A micro-combination glass pH electrode (Microelectrodes, Model M1-410) was used to measure the pH of individual rain droplets on the surface of leaves. This electrode was used in conjunction with an Orion Model 801 digital pH meter and gave precise readings of pH with an almost immediate response time. Droplets of simulated rain solution ('B' rain solution) were applied in equal numbers to leaves of intact plants using a micropipette which delivered 50 µl of solution. A single droplet was measured for pH only once to prevent any pH changes that could occur from leakage of KCl from the electrode. Thus, data for changes that occurred in droplet pH with time were obtained by measuring droplets randomly on different leaves of 5 replicate plants. Three droplets were placed on each leaf and the mean $[H^+]$ for each leaf was used. Droplets placed on strips of parafilm served as controls for changes in pH due to evaporation. When the temperature was maintained at about 22°C it took approximately 80 minutes for rain droplets to dry on the leaf surface.

B. Elemental analysis of foliage

Leaves of *A. tilesii* were analyzed after one month of simulated rain treatments of pH 5.6, 4.0, 3.0, 2.5 and 2.0 for elements likely to be leachable under acidic conditions. A Wiley Mill, with a stainless steel hopper and sieve, was used to grind leaves to <1.0 mm, prior to acid digestion. Chemical elements in plant material were solubilized by digestion in hot, concentrated nitric acid (Allen et al, 1974). In early trials hydrogen peroxide was added in the latter stages of the digestion procedure, but comparisons showed that suitable results were achieved using only nitric acid. All glassware was acid washed and then rinsed throughly with deionized-distilled water before use. Each batch of plant digests was accompanied by 3 reagent blanks and 3 plant material "standards" (U.S.N.B.S. orchard leaves).

Elemental analysis was performed on an atomic absorption spectrophotometer, Perkin-Elmer 460, using hollow cathode lamps for Ca, Mg and Na and on emission mode for K. Absorbance readings were compared to those for standards, which were prepared from certified atomic absorption standard solutions (Fisher). Samples were diluted (1:100) prior to reading in order to obtain concentration readings within the linear portion of the optical absorbance-element concentration curve. Samples which were analyzed for Ca and Mg received an addition of 0.4% lanthanum (as $LaCl_3$) to control ionization of the element in the flame (Allen et al, 1974; Perkin-Elmer Corp., 1976). Calcium analysis is subject to interferences from other elements present in the sample solution (Allen et al, 1974). Samples which were analyzed for K and Na received an addition of 0.1% NaCl and 0.04% KCl, respectively, to overcome ionization of these elements in the air acetylene flame (Allen et al, 1974; Perkin-Elmer Corp., 1976). Concentrations of elements in plant material were determined to within 9, 14, 6 and 18%, for Ca, Mg, Na and K respectively, in certified samples of commercially available orchard leaves.

C. Soil pH determination

Soil was pooled for the control and each acid treatment and sub-samples taken to determine if changes had occurred in soil pH due to rainfall applications. pH determinations were made on soil samples to which an equal volume of deionized-distilled water was added to produce a slurry. After mixing thoroughly, this slurry was allowed to equilibriate for approximately 1 hour before the pH was determined using a combination electrode and a Fisher Accumet 310 pH meter.

Data handling and statistical tests

Statistical analyses were carried out using the University of Toronto's IBM 3033-N8 Computer (MVS-JES 2 operating system) and the Statistical Analysis System (Barr *et al*, 1976) and Statistical Package for the Social Sciences (Nie *et al*, 1975) statistical package programs (SAS and SPSS). Analyses of variance, 2 and 3-way, (Sokal and Rohlf, 1969) were used to determine the between treatment effect (including pH, number of exposures, rainfall duration, frequency of rainfall exposure, species and genotype) and between replicate effects for percentage of leaf area injured, dry weight, number of leaves produced, cell sizes of various leaf tissues, leaf chlorophyll content, and foliar concentration of various elements. All measurements of growth were subjected to log transformation prior to analysis (Sokal and Rohlf, 1969). If the results of an analysis of variance (ANOVA) proved significant, then Duncan's Multiple Range tests were used to identify which means differed significantly ($P<0.05$) from each other.

The results of an experiment, in which foliar injury (expressed as the percentage of leaves injured) of different genotypes of *Artemisia tilesii* was examined over a four week period of exposure to acidic rain, were analysed using a multivariate analysis of variance (MANOVA). Multivariate analysis of variance was appropriate since it allowed the differences between the clones to be compared over the whole course of the experiment rather than at individual points in time. The percentage injury data were subjected to an arcsin $\sqrt{\ }$ transformation before analysis (University of Toronto Statistical Consulting Service, personal communication). The results of the experiment in which buffering capacity (concentration of H^+ neutralized) of different species was measured was also analysed using multivariate analysis of variance. This test allowed the differences between species to be compared over the 75 minute period of droplet pH measurements.

Average pH values were calculated by converting to the equivalent hydrogen ion concentration to obtain the mean and then converting back to pH. pH is used only as a matter of convenience since this scale is more familiar to most people. However, the reader should bear in mind that the pH scale is logarithmic and thus small changes at the low end of the scale are significant. For this reason pH changes occurring in droplets on leaf surfaces are also accompanied in the thesis by values of the equivalent change in hydrogen ions and the standard deviation of the hydrogen ion concentration.

Calculations for the frequency with which lesions occurred over vascular tissue, including the assumptions involved, are outlined in Appendix 5.

RESULTS

Foliar injury

A. Macroscopic effects

1. Visual injury symptoms

Visual symptoms of foliar injury were observed on plants where the acidity of simulated rainfall was 3.0 or below. Small necrotic lesions (pitting) appeared at pH 3.0 and larger areas of bifacial necrosis occurred at pH 2.5 and 2.0. The lesions, which were light golden-brown patches, were scattered over leaves, giving them a blotchy appearance. This description of visual injury symptoms generally held for the more than 600 _Artemisia_ and 150 bean and spinach plants treated during the 2 year period of experimentation, although the amount of injury varied for different species. These same symptoms were produced regardless of whether the simulated rainfall contained additions of cations and anions, and was adjusted with a 2.5:1 molar ratio of sulphuric:nitric acid, or whether it was simply distilled water adjusted to the desired pH with sulphuric acid. Recently, similar injury has been described for a number of crop plants and tree species (Shriner _et al_, 1975; Ferenbaugh 1976; Hindawi _et al_, 1977; Jacobson and Leuken, 1977; Evans _et al_, 1978 and Evans, 1982). Photographs taken of typical acid rain damage (Plates 1 - 3) show injury to foliage of _Artemisia tilesii_, _Phaseolus vulgaris_ (wax bean) and _Spinacea oleracea_ (spinach).

Development of lesions generally occurred within 12 - 24 hours of treatment. For treatments of extreme acidity (pH 2.0) however, damage to the leaf surface was visible at the locations of droplet accumulation even before they had completely dried. Initially the leaf surface was bronze in colour and had numerous pitted areas, which eventually became the typical light-brown necrotic lesions.

No morphological or developmental abnormalities, such as excessive branching, stunting or changes in leaf size, occurred to treated plants. If severe acidic rainfall treatments (pH 2.0) were applied over a longer period of 2 - 4 weeks, however, chlorosis, as well as necrosis was observed, and in some instances, leaves died. Premature leaf abscission occurred frequently for plants of two Artemisia clones (Smoking Hills # 3 and 9) which developed necrosis at nodal regions. Both of these clones had vertically-oriented leaves, which clasped the stem at the leaf base, allowing solutions to pool in the nodal areas. Young Artemisia leaves were frequently burned at the leaf tip and margins. This injury occurred while the young developing leaves were still folded in the shoot apex, as a result of acidic droplets adhering to the shoot tip. This would appear to be a sensitive stage in leaf development to acid rain injury.

2. Effects of pH and chemical composition of simulated rainfall

a) pH effects

Foliar injury (lesions) to Artemisia occurred when the rainfall pH was 3.0 or below. All of the experiments were conducted with mature Artemisia individuals propagated from cuttings. Previous studies have suggested that seedlings may be more sensitive to acid rain than older plants (Evans et al, 1977a; Wood and Bormann, 1977). The number of leaves injured (Figure 2) and the total area of leaf surface injured (Table 12) increased as the pH of 'rain' was decreased and with

Figure 2 The effect of simulated rain on the percentage of leaves injured to *Artemisia tilesii* (Smoking Hills clones).

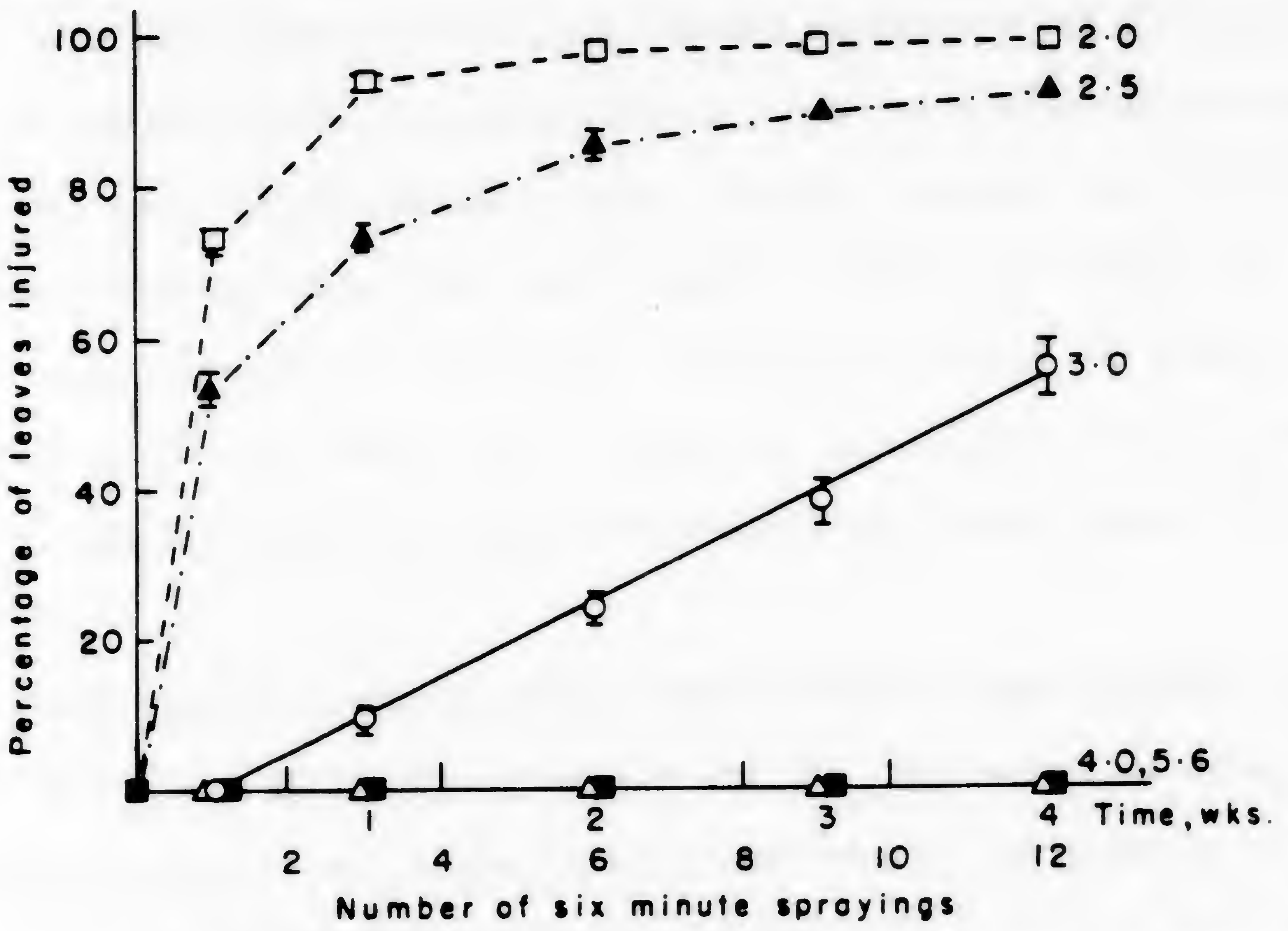


Table 12 The effect of simulated acid rain on the percentage of leaf area injured* in *A. tilesii* (Smoking Hills clones).

pH	N	Percentage of leaf area injured*	
		6 sprays $\bar{x} \pm$ SD	12 sprays $\bar{x} \pm$ SD
5·6	60	0·0 ± 0·0	0·0 ± 0·0
4·0	60	0·0 ± 0·0	0·0 ± 0·0
3·0	60	1·4 ± 1·3	2·3 ± 1·5
2·3	60	4·1 ± 3·8	6·5 ± 9·3
2·0	60	33·7 ± 20·2	45·8 ± 27·0

* Simulated rain events were 6 minutes long and given 3 times weekly.

repeated exposures of acidic rain. The amount of foliar injury induced by rain of pH 3.0 was small (<3% of the leaf surface after 12 sprays), for all of the 9 clones tested from the two populations, and in most instances lesions did not occur until several exposures at this pH. An increase in the amount of leaf area injured occurred at pH 2.5 (6.5% of the leaf surface after 12 sprays), while at pH 2.0 severe foliar injury occurred, with lesion damage (from either necrosis or chlorosis) affecting almost 50% of the leaf surface (Table 12, Figure 3). A significant reduction in the total chlorophyll content of leaves was observed at pH 2.0 (Table 13). Losses of chlorophyll a were greater than b, which altered the mean chlorophyll a:b ratio from 2.7:1 to 2.3:1.

These results indicate that, while lesions were produced on a large percentage of leaves at pH 2.5 and 3.0, the amount of damage to Artemisia leaves is not extensive until the rain pH falls below 2.5.

b) Rainfall chemistry effects

During initial experiments the simulated acid rain solutions were prepared by adjusting distilled water to the desired pH with sulphuric acid. The results from these experiments, both in the injury symptoms produced and their extent, did not appear to differ from those of later experiments in which the rain was prepared to more closely simulate ambient rainfall. This was done by adding cations and anions to the rain solution (see Methods) and adjusting the pH with a 2.5:1 molar ratio of sulphuric:nitric acid. These results would suggest that the hydrogen ion concentration of the rainfall is the major factor determining foliar response, other chemical constituents having an insignificant effect at the concentrations used.

Figure 3 Tracings of the amount of leaf surface area injured for two Smoking Hills clones of A. tilesii exposed to acidic rain for a period of 4 weeks (12 six min. rainfalls). (Note differences in leaf morphology of clones 8 and 9.)

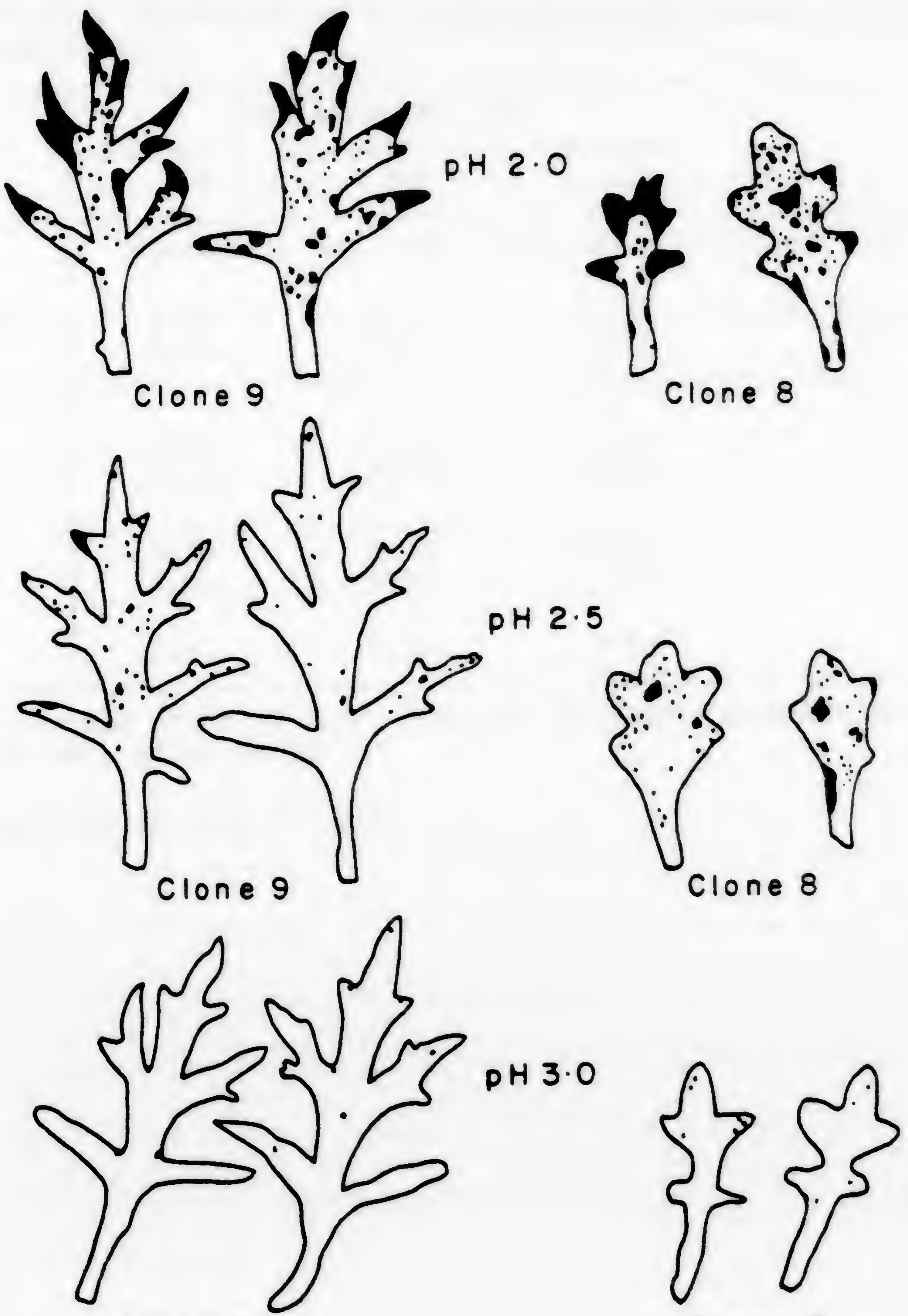


Table 13. Chlorophyll concentration in *Artemisia tilesii* leaves exposed to simulated acid rain.[a]

Chlorophyll	Simulated Rain pH				
	5.6	3.0	2.0		
	Chlorophyll (mgs./gm. fresh wt.)[b]			F Value	PR>F
Total Chlorophyll	1.08 ±.03	1.22 ±.19	0.53 ±.22	19.14	P<0.001
Chlorophyll *a*	0.78 ±.02	0.89 ±.16	0.36 ±.18		
Chlorophyll *b*	0.30 ±.02	0.33 ±.03	0.16 ±.06		
Ratio *a*:*b*	2.6:1	2.7:1	2.3:1		

[a] Leaves were analyzed after 10 days, with one 20 minute exposure to simulated acid rain given daily.

[b] Mean of 4 plant replication ± 1 S.D.

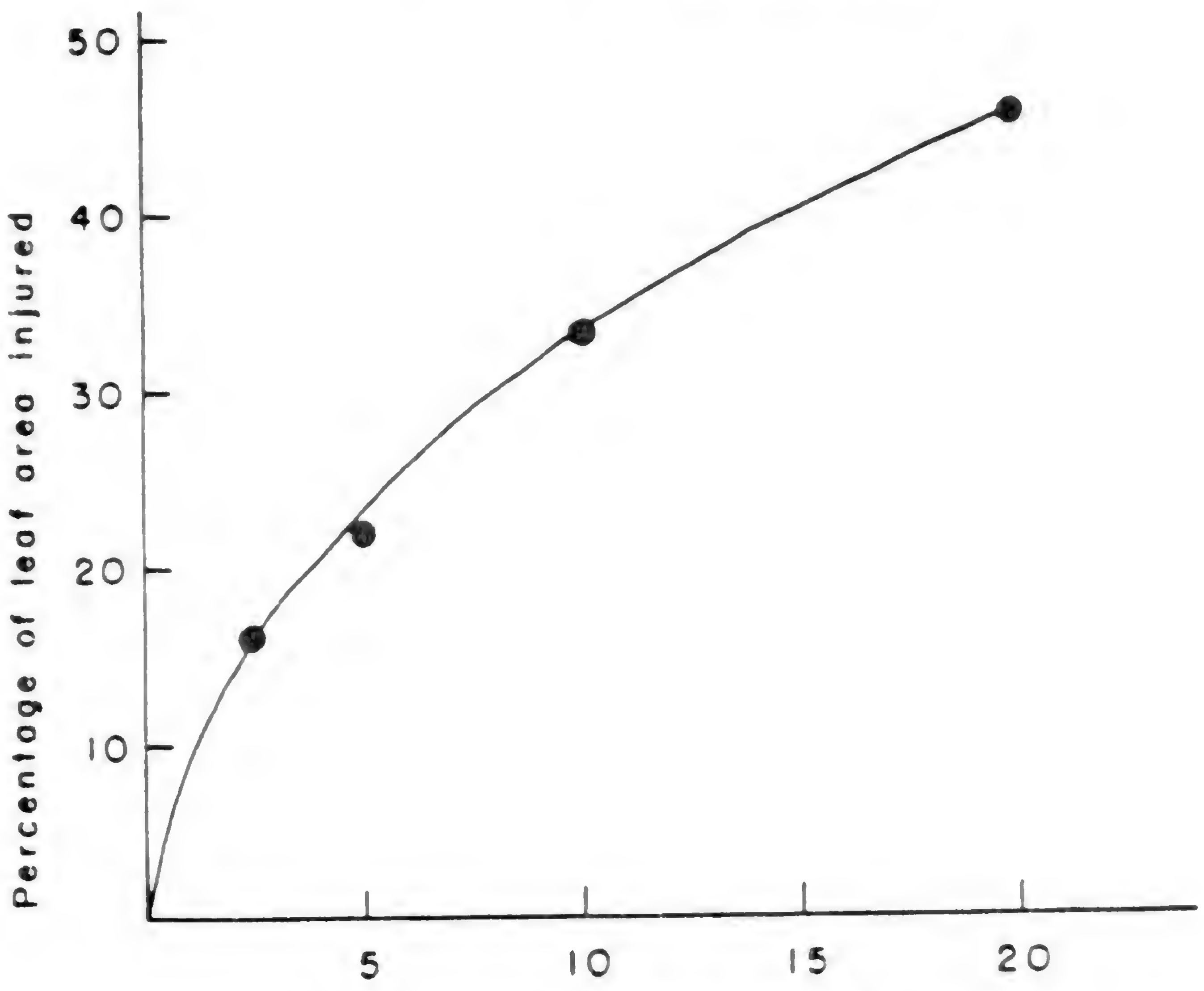
3. Effects of rainfall duration and frequency

Foliar response was altered by differences in the duration of rainfall exposure and by changes in the length of time between treatments. The treatments used ranged in duration from 2.5 - 20 minutes and were applied, either on consecutive days for 10 days, or every other day for 20 days to *Artemisia tilesii* (Smoking Hills clones 5 and 8).

The results of an analysis of variance of the percent of leaf area injured (Table 15) when the pH was 2.0 showed a highly significant effect ($P<0.001$) of rainfall duration and a significant interaction between rainfall duration and frequency. However, there was no significant effect due to the frequency of rainfall ($P<0.05$, Table 16b). The percent of leaf area injured doubled when the rainfall duration was extended from 2.5 to 10 minutes, and tripled when increased to 20 minutes (Table 15, Fig. 4, Plate 6). Although the percent of leaf area injured increased dramatically as the duration of treatment was lengthened, there was no similar effect on the percent of leaves which were injured (Table 14). This is explained however by the fact that for even the shortest exposure treatment of 2.5 minutes, nearly 100% of the leaves showed acid injury at pH 2.0.

Under identical regimes of rainfall duration and frequency, a treatment of pH 3.0 gave very different results. In this instance rainfall frequency proved to have a significant effect on the percent of leaf area injured, while there was no significant effect due to rainfall duration and no significant interaction between these two factors ($P<0.05$, Table 16a). The results for the percent of leaves injured showed a similar pattern to the percent of leaf area injured,

Figure 4 Effect of the duration of rainfall exposure (pH 2·0) on the percent of leaf area injured.* (Smoking Hills, *A. tilesii*)



* Leaf area injured after 10 exposures.

Plate 6 Photograph of *Artemisia tilesii* plants (Smoking Hills) showing increasing foliar injury as the length of rain exposure is increased from 2·5 to 20 minutes.

Table 14. Effect of rainfall duration and frequency on the percent of *Artemisia tilesii* (Smoking Hills) leaves injured by simulated acid rain[a].
(Each value represents the mean of 6 plants).

	% of acid injured leaves by pH and exposure duration (mins)							
	pH 2.0				pH 3.0			
Spray Frequency	2.5	5	10	20 min	2.5	5	10	20 min
Sprayed every day	94.5	99.3	97.3	99.5	74.5	76.2	59.2	66.2
Sprayed every other day	100.0	100.0	100.0	100.0	25.2	31.3	22.2	24.7

[a] Percent of leaves injured after 10 exposures; data for foliar injury estimates made for fewer exposures is given in Appendix 1.

NOTE: No injury to plants treated with pH 5.6.

Table 15. Effect of rainfall duration and frequency on the percent of leaf area injured[a], at pH 2.0 and 3.0 to *Artemisia tilesii* (Smoking Hills).
(Values are means of 6 plants, 3 leaves each ± 1 SE)

	% of leaf area injured by rainfall duration and frequency			
	2.5	5	10	20 min
pH 2.0 Sprayed every day	22.2 ± 4.2	26.0 ± 5.5	24.4 ± 3.6	41.6 ± 6.4
Sprayed every other day	10.0 ± 1.5	17.8 ± 2.4	42.2 ± 5.8	48.9 ± 4.2
pH 3.0 Sprayed every day	2.2 ± 0.2	1.8 ± 0.3	1.7 ± 0.3	1.4 ± 0.3
Sprayed every other day	1.4 ± 0.2	1.1 ± 0.3	1.1 ± 0.3	1.4 ± 0.3

[a] Leaf area injured after 10 exposures.

Table 16a. The effect of rainfall duration and frequency on the percent of leaf area injured (pH of simulated rain = 3.0): ANOVA results.

Source of Variation	DF	Anova SS	F
		Analysis of variance	
Frequency	1	10.49	7.21**
Duration	3	4.68	1.07
Frequency*Duration	3	2.93	0.67

***=$p<0.001$ **=$p<0.01$

Table 16b. The effect of rainfall duration and frequency on the percent of leaf area injured (pH of simulated rain = 2.0): ANOVA results.

Source of Variation	DF	Anova SS	F
		Analysis of variance	
Frequency	1	38.91	0.11
Duration	3	17995.17	16.42***
Frequency*Duration	3	5302.72	4.84**

***=$p<0.001$ **=$p<0.01$

Duncan's Multiple Range test for duration of rainfall - means with the same letter are not significantly different ($\alpha=0.05$).

Grouping	% Leaf area injured* $\bar{x}$	Spray duration (min)
A	45.5	20
B	33.3	10
C	21.9	5
C	16.1	2.5

* Calculated by averaging the values from plants sprayed daily and those sprayed every other day.

at pH 3.0 (Table 14). One-third as many leaves were injured on those plants treated on alternate days as for those treated daily, after 10 exposures, while there were no differences in the percent of leaves injured for rainfalls of varying duration.

The dose-response experiments with Artemisia indicate that rainfall duration and frequency are both important criteria in determining foliar injury. The exact nature of the response is complex, however, and dependent on the acidity of the rainfall. At acidity levels which cause little injury to the foliage, rainfall frequency appears to be more important in determining the response, while at acute acidity levels it appears that the duration of exposure may be the more important factor in determining the amount of foliar injury.

4. Foliar response of *Artemisia tilesii* from an acid site(Smoking Hills) and a control site (Alaska)

Experiments were performed to study the effect of simulated acid rain on the foliar responses of clones of Artemisia from the Smoking Hills, where this species survives under conditions of extreme SO_2 and acid aerosol exposure, and from a control site in Alaska. Initially, the responses of 6 clones from the Smoking Hills population to simulated acid rain, were examined to determine the degree of variation within the acid-stressed population. Three of these clones, which were determined to have the greatest variation in sensitivity to foliar injury, were also used in a succeeding experiment designed to compare the foliar and growth responses of Smoking Hills clones with clones from the Alaskan 'control' population. Due to the small number of clones available for the 'between populations' comparison (3 clones from each population), it is recognized that further studies using additional clones are necessary before any firm conclusions can be drawn as to whether ecotypic differentiation has occurred in Artemisia in response to increased acidity at the Smoking Hills.

The foliar response of Artemisia clones was measured in two ways: (a) the percent of acid injured leaves and (b) the percent of leaf area injured, which was estimated using a modified Horsfall-Barratt (Horsfall and Barratt, 1945) ranking scale (Table 9). No fewer than 10 plants were scored for each clone for a given pH treatment.

a) Foliar injury to six clones from the Smoking Hills population

The production of foliar lesions by simulated acid rain varied amongst the 6 clones from the Smoking Hills population. The percentage of leaves injured on individuals was recorded at regular intervals throughout the 4 week period during which plants received 12 exposures to simulated rain (pH 5.6, 4.0, 3.0, 2.5, 2.0). These injury values (Appendix 2) were subsequently transformed, in order to produce homogeneous variances, using an arcsin $\sqrt{}$ transformation and then the injury curves over time were analyzed using a multivariate analysis of variance (MANOVA) to test for differences between the clones. Multivariate analysis of variance indicated significant differences in the foliar response of the 6 clones to simulated acid rain, at pH 2.0, 2.5 and 3.0 ($P<0.01$, Table 17). None of the clones were injured at pH 4.0 or 5.6. Duncan's Multiple Range test was applied to the foliar injury data for the 6 clones for each of the three significant pH treatments (Table 18). Among the clones three groups (arranged from the most sensitive to the least sensitive to foliar injury) were consistently present at each of the 3 pH treatments where foliar injury was induced:

I. Most susceptible to foliar injury . . . . . . Clones 9 and 3
II. Intermediate susceptibility to foliar injury. Clones 8 and 5
III. Least susceptible to foliar injury. . . . . . Clones 11 and 12

The foliar response pattern for clones from each of the three groups is shown in Figures 5a, b, c. For each response curve, one line has been drawn to fit the injury values from two clones which showed nearly identical levels of injury, with the exception of clones 11 and 12 at pH 3.0. It should be noted that while the shape of the response curves differs depending on the pH, the same pairs of clones follow the same ordering of susceptibility to foliar injury at pH 2.0, 2.5 and 3.0.

The differential sensitivities of the 6 clones was assessed on the basis of their individual morphology (plant stature, leaf orientation, leaf shape and size, amount of leaf dissection etc.). This was possible due to the large phenotypic differences between individual clones in the population (See Fig. 3 for extremes in leaf morphology). However, cuttings taken from an individual clone resembled the parent clone quite closely. Qualitative observations of morphological parameters showed a strong morphological resemblance in pairs of clones which responded similarly in the percent of leaves injured by acidic rain (Clones 9 and 3; 8 and 5; 11 and 12). Clones 9 and 3, which showed the greatest sensitivity to foliar lesions, were the only two clones which had a trailing growth habit, all of the others being upright. The leaves of clones 9 and 3 were very long and broad and

Table 17. Manova tests for the effect of clones on the percentage of injured leaves of Artemisia tilesii.

pH of rainfall	Test statistic	F Values[a]
3.0	Hotelling-lawley trace	5.77 ***
	Pillai's trace	2.97 ***
	Wilks' criterion	4.21 ***
2.5	Hotelling-lawley trace	4.18 ***
	Pillai's trace	2.77 ***
	Wilks' criterion	3.48 ***
2.0	Hotelling-lawley trace	2.97 ***
	Pillai's trace	1.84 **
	Wilks' criterion	2.37 **

[a] Significance level: *P<0.05 **P<0.01 ***P<0.001

NOTE: An arcsin $\sqrt{\ }$ transformation was applied to the percentage of leaves injured prior to the multivariate analysis of variance.

Table 18. Differences in the percentage of leaves injured for six clones of *Artemisia tilesii* from the Smoking Hills population. (Injury was measured after 12 six minute rainfalls)

pH 3.0 rainfall	A	A	B	B	C	C
$\bar{x}$ % leaves injured[a]	68.1	64.5	49.1	47.6	36.3	28.9
	SH#9[b]	SH#3	SH#8	SH#5	SH#12	SH#11
pH 2.5 rainfall	A	A	AB	BC	CD	D
$\bar{x}$ % leaves injured	83.9	83.0	78.9	74.7	69.7	67.0
	SH#3	SH#9	SH#5	SH#8	SH#12	SH#11
pH 2.0 rainfall	A	A	A	A	A	B
$\bar{x}$ % leaves injured	90.0	88.8	88.2	87.4	87.2	82.5
	SH#9	SH#3	SH#11	SH#8	SH#5	SH#12

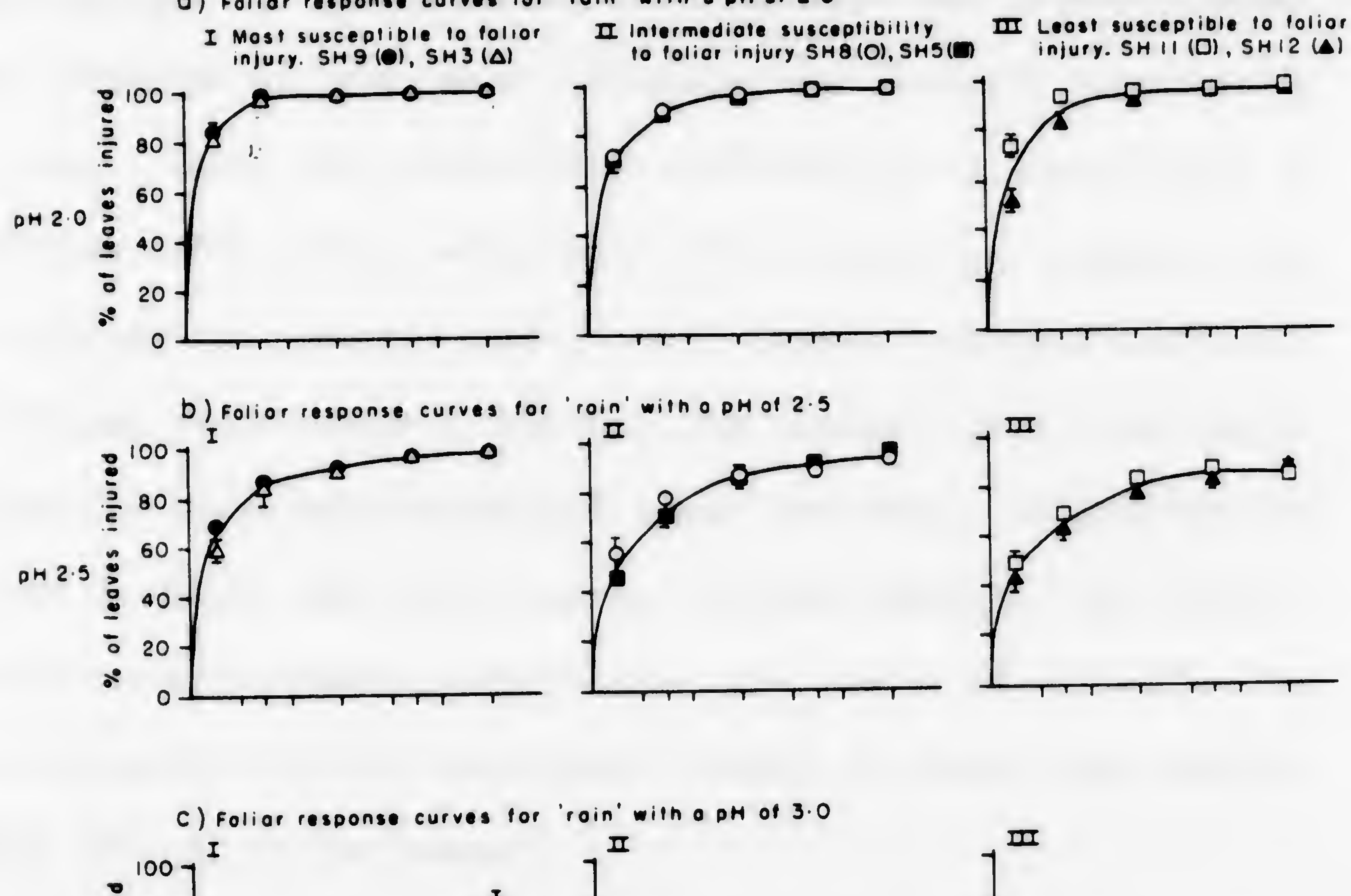
[a] Injury values after an arcsin $\sqrt{}$ transformation.

[b] Smoking Hills (SH) clones 3, 5, 8, 9, 11, 12 were used in this experiment.

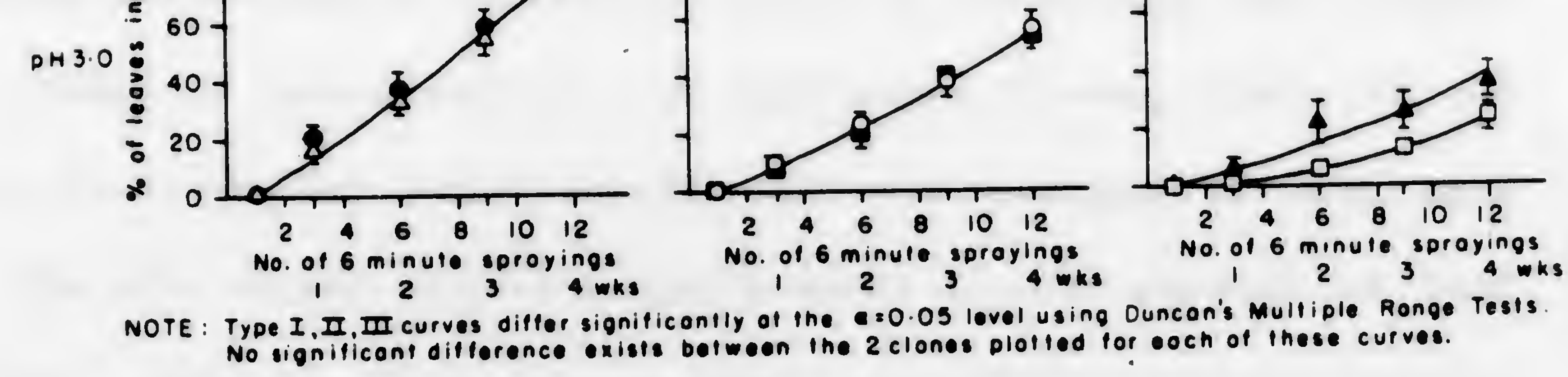
NOTE: Means having the same letter do not differ significantly ($\alpha = 0.05$, Duncan's Multiple Range Test).

Figure 5 (a–c) Effect of pH on the percentage of leaves injured for six clones from the Smoking Hills 3 patterns of response. (Values are mean of 10 plants ± 1 S.E. Where S.E. is absent it is < the diameter of the symbol used or is zero.)

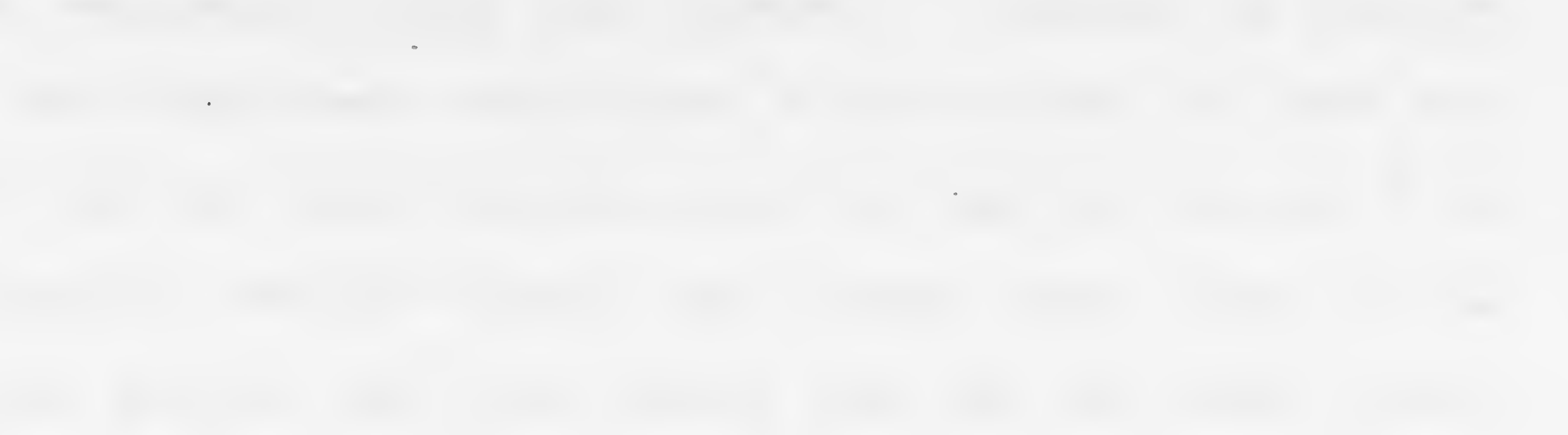
a) Foliar response curves for 'rain' with a pH of 2·0



b) Foliar response curves for 'rain' with a pH of 2·5



c) Foliar response curves for 'rain' with a pH of 3·0



NOTE: Type I, II, III curves differ significantly at the α=0·05 level using Duncan's Multiple Range Tests. No significant difference exists between the 2 clones plotted for each of these curves.

were produced at the furthest intervals from each other along the stem, of any of the other clones. Because of this, rainfall had a greater probability of wetting all or most of the leaves on a plant. The leaf orientation also differed from that of other clones. Leaves clasped the stem at the leaf base and were oriented upwards, which tended to favour the pooling of water, particularly in the nodal area. Clones 5 and 8 were upright in growth habit, with smaller but broader leaves which were produced at very short intervals and extended horizontally from the stem. These two clones were intermediate in sensitivity to foliar lesions. The clones which were least sensitive, clones 11 and 12, were upright in growth habit, with longer, narrower and more dissected leaves than clones 5 and 8. Like clones 5 and 8 the leaves were produced at very short intervals along the stem. In contrast to clones 9 and 3, which had their leaves oriented upwards, and clones 5 and 8, which extended theirs horizontally, the leaves of clones 11 and 12 curved downwards at their ends which tended to favour the droplets rolling down and off of the leaves.

Although morphological parameters could be correlated very closely with the percent of leaves injured for individual clones, there did not appear to be a similar relationship with the percent of leaf area injured. The six clones varied significantly in the percent of leaf area injured. However, there was no clear correlation between similar clones and magnitude of leaf area injured. Analyses of variance revealed that significant differences in the percent of leaf area injured for the six clones occurred at pH 2.0 and 2.5, but not at pH 3.0. Duncan's Multiple Range test showed that clone 8 had substantially greater leaf area injured than did the other clones, which did not differ significantly from each other, at pH 2.5 (Table 19). At pH 2.0 clone 8 also had the greatest amount of leaf area injured. However, there was more variability in the foliar response of the six clones than occurred at pH 2.5 (Table 19). It is interesting to note that clones 9 and 3 had the least injury of all of the clones when ranked according to the percent leaf area injured at both pH 2.5 and 2.0, yet these two clones were the most sensitive on the basis of the percent of leaves which were injured.

While plant morphology is important in determining the number of leaves which sustain injury in the form of lesions from acid rain, it appears that other characteristics of leaves are involved in determining the area of leaf surface injured. Individual clones from the Smoking Hills population varied in their response to simulated acid rain. This suggests that the degree of phenotypic variation has a bearing on how foliar injury should be assessed. In comparing the foliar responses of plants which are variable morphologically, it would be more relevant to use the percent of leaf area injured to measure the response, particularly if one is interested in making comparisons of the sensitivity of different species. The amount of leaf area injured would also have greater significance in terms of expected growth effects.

b) Comparison of foliar injury to clones from two populations

The effects of simulated acid rain on the foliage of 3 clones from the Smoking Hills was compared with 3 clones from the Alaska 'control' population. Necrotic lesions were produced on the foliage of clones from both populations with treatments of pH 2.5 and 3.0. Clones from

Table 19. Percent leaf area injured for Artemisia clones from the Smoking Hills.
(Values are means for ten plants ± 1 S.D.)[a]

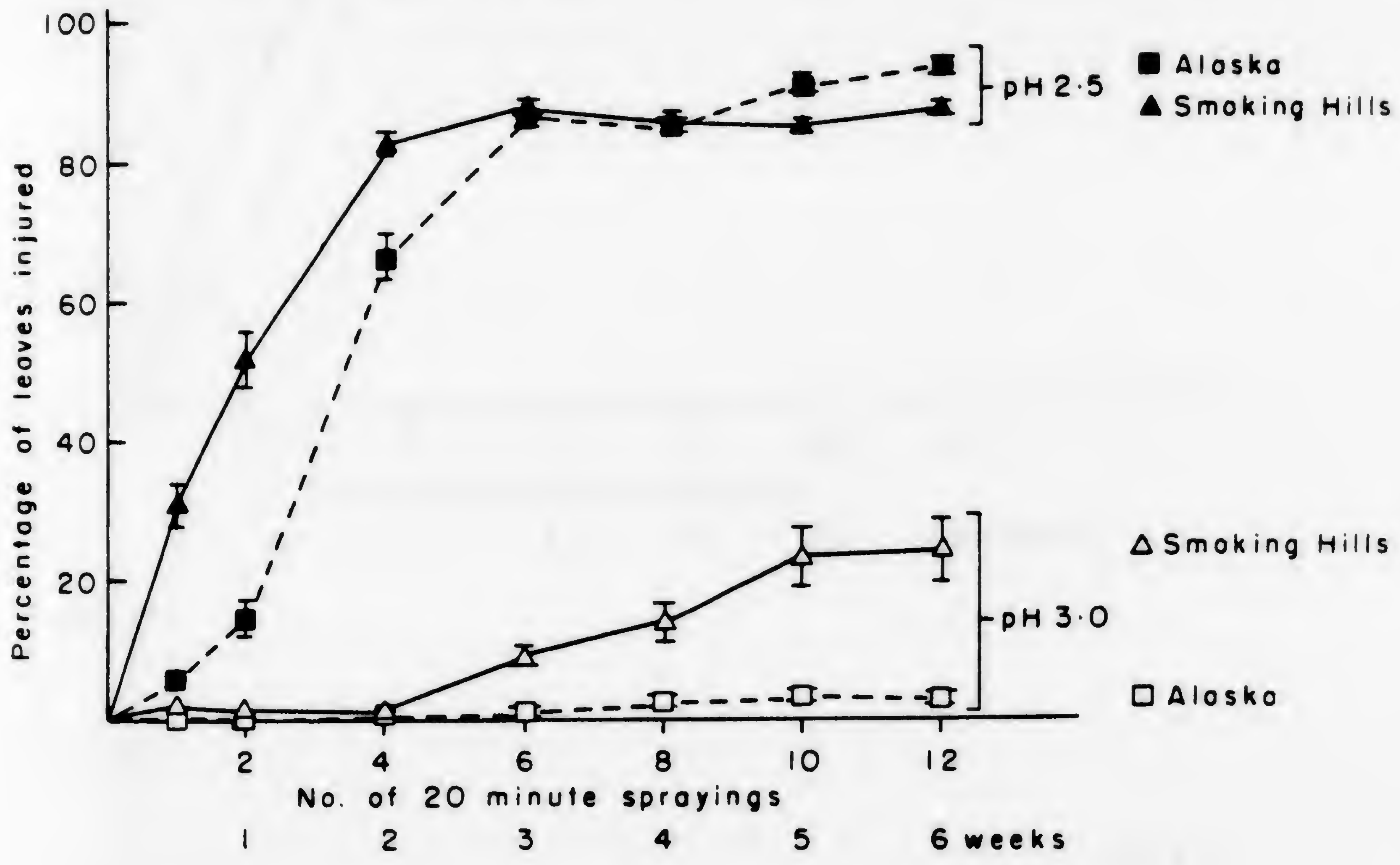
pH	Clone	6 Sprays (2 weeks)[b] $\bar{x}$ ± S.D.	12 Sprays (4 weeks) $\bar{x}$ ± S.D.	
2.0	8	40.6 ± 28.0	58.1 ± 33.4	A[c]
	5	36.5 ± 16.5	54.8 ± 22.1	A
	11	39.6 ± 26.1	53.1 ± 28.2	A
	12	35.0 ± 17.4	46.3 ± 29.6	AB
	3	26.2 ± 10.1	33.5 ± 14.9	BC
	9	26.8 ± 15.9	28.3 ± 17.3	C
Grand Mean		33.7 ± 20.2	45.8 ± 27.1	
2.5	8	6.2 ± 3.9	13.7 ± 17.8	A
	5	3.3 ± 3.0	6.0 ± 7.6	B
	11	3.7 ± 3.1	6.7 ± 7.8	B
	12	3.5 ± 2.1	3.5 ± 2.0	B
	3	3.7 ± 2.1	5.2 ± 4.1	B
	9	3.2 ± 1.7	3.5 ± 2.0	B
Grand Mean		4.1 ± 3.8	6.4 ± 9.2	
3.0	8	1.8 ± 1.1	2.7 ± 2.9	A
	5	1.6 ± 1.2	2.2 ± 0.9	A
	11	1.0 ± 1.3	1.6 ± 1.2	A
	12	1.5 ± 1.3	2.2 ± 0.9	A
	3	1.5 ± 1.3	2.3 ± 0.6	A
	9	0.9 ± 1.2	2.5 ± 0.0	A
Grand Mean		1.4 ± 1.3	2.3 ± 1.5	
4.0		No injury occurred to any of the six clones		
5.6		No injury occurred to any of the six clones		

[a] Injury was estimated for 3 fully expanded leaves on each plant using 10 categories of percent leaf area injured: 0%, 1 - 5%, 6 - 10%, 11 - 25%, 26 - 50%, 51 - 75%, 76 - 90%, 91 - 95%, 96 - 99%, 100%.

[b] Tests of significance were applied only to injury values for 4 weeks.

[c] Means followed by the same letter are not significantly different (α =0.05, Duncan's Multiple Range test).

Figure 6 Foliar injury to *Artemisia tilesii* from the Smoking Hills and Alaska (n = 40).



* No injury occurred to plants treated with pH 3·5, 4·0 and 5·6.

Figure 7 Sensitivities of Smoking Hills clones to simulated acid rain. (n = 14 replicates / clone).

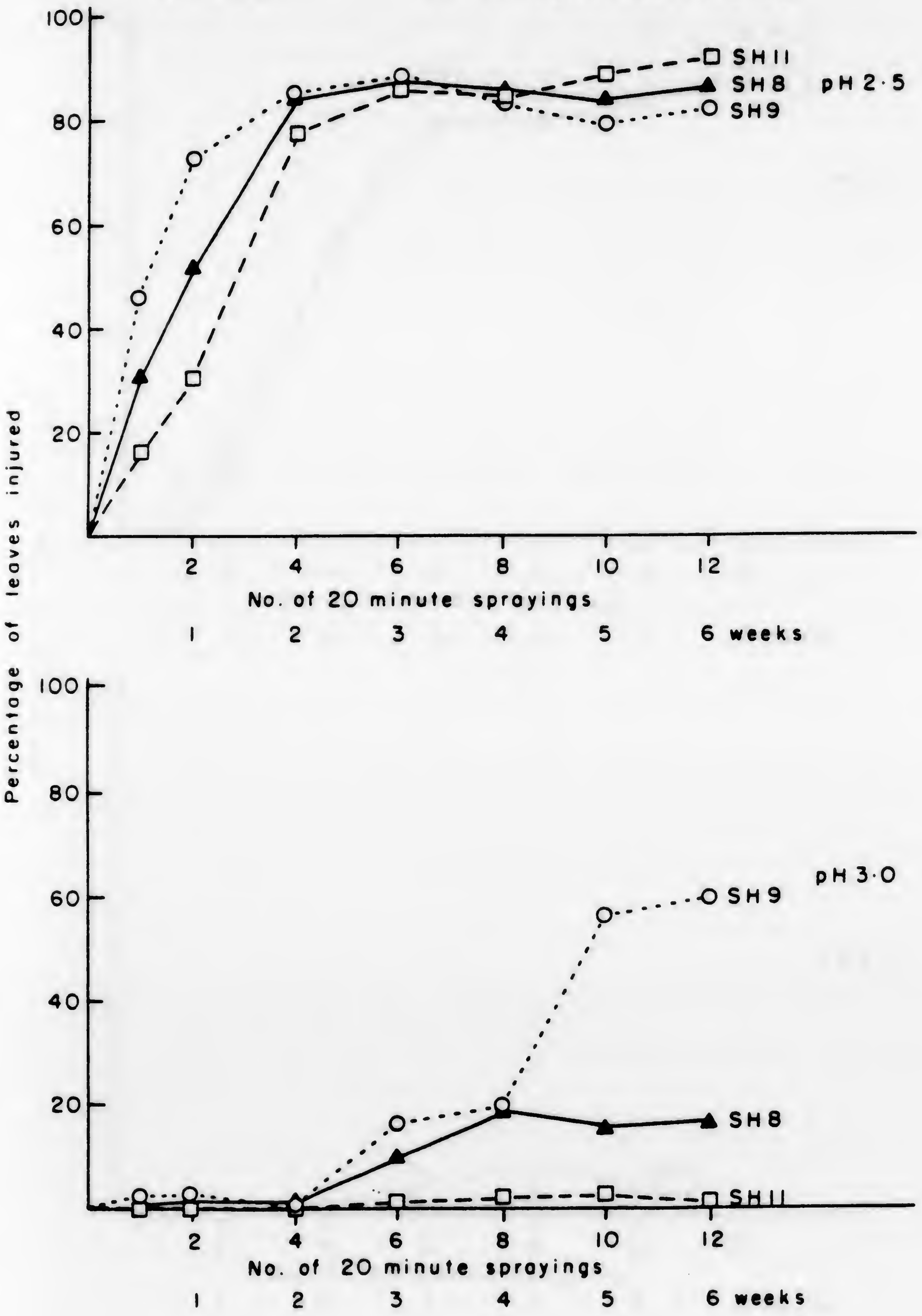


Figure 8 Sensitivities of Alaskan clones to simulated acid rain.
(n=14 replicates / clone)

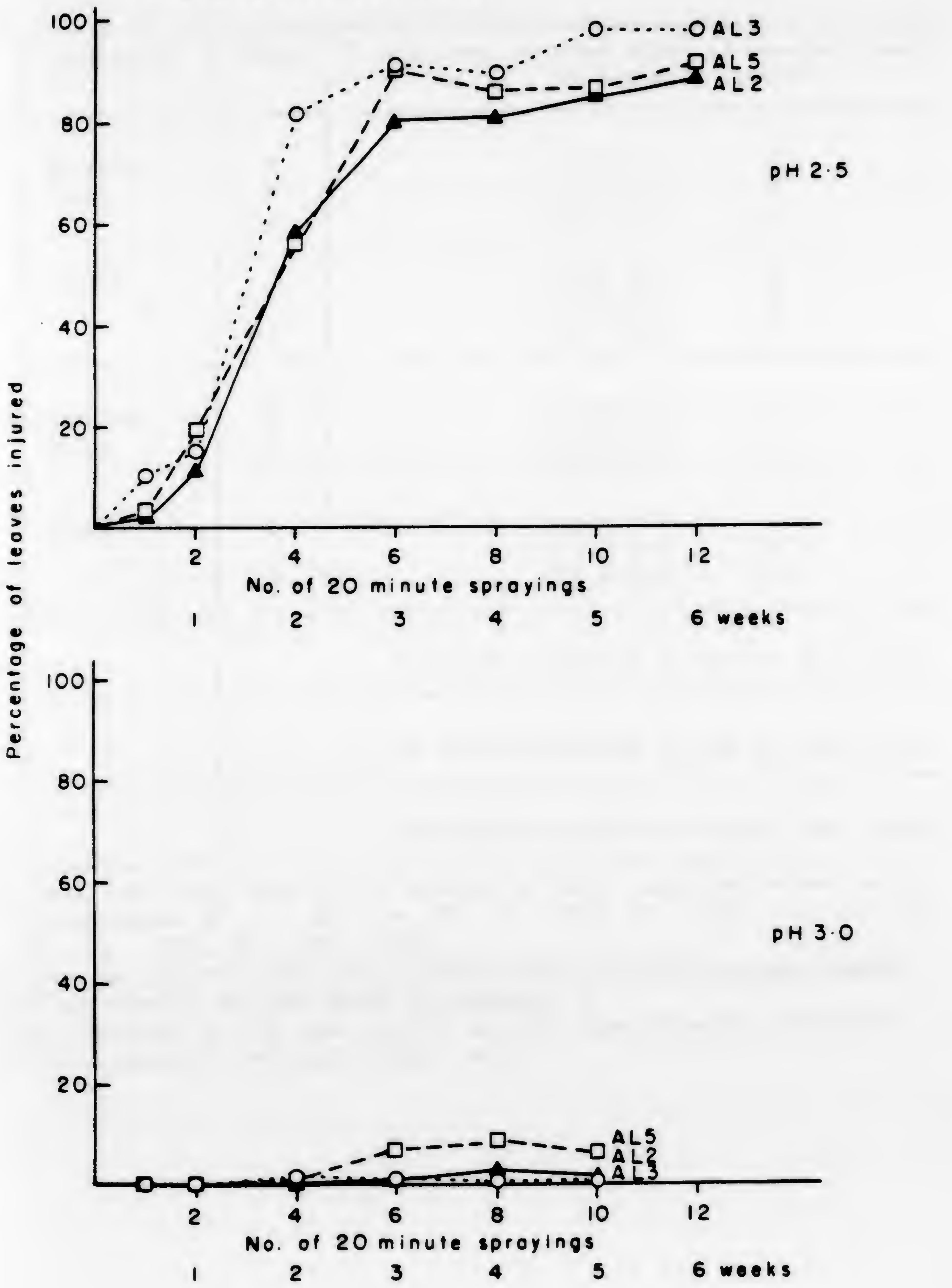


Table 20. Percent leaf area injured for Artemisia clones from the Smoking Hills and from Alaska.
(Values are means for fourteen plants ± 1 S.D.)[a]

pH	Population	Clone	% leaf area injured after 12 sprays(6 wks)[b]	
			$\bar{x}$ ± S.D.	α=0.05
2.5	Smoking Hills	9	9.0 ± 4.6	A[c]
		8	4.6 ± 4.3	B
		11	3.6 ± 2.1	B
	Alaska	5	4.2 ± 2.5	B
		3	2.3 ± 0.7	B
		2	2.1 ± 0.9	B
3.0	Smoking Hills	9	1.5 ± 1.3	A
		8	1.6 ± 1.2	A
		11	0.5 ± 1.1	B
	Alaska	5	0.5 ± 1.0	B
		3	0.0 ± 0.0	B
		2	0.0 ± 0.0	B
3.5		No injury occurred to any of the clones		
4.0		No injury occurred to any of the clones		
5.6		No injury occurred to any of the clones		

[a] Percent leaf area injured was scored on fully expanded leaves using 10 categories: 0%, 1 - 5%, 6 - 10%, 11 - 25%, 26 - 50%, 51 - 75%, 76 - 90%, 91 - 95%, 96 - 99%, 100%.

[b] Values for percent leaf area injured after 18 and 24 sprays showed similar results and are given in Appendix 4.

[c] Means followed by the same letter are not significantly different (α=0.05, Duncan's Multiple Range test).

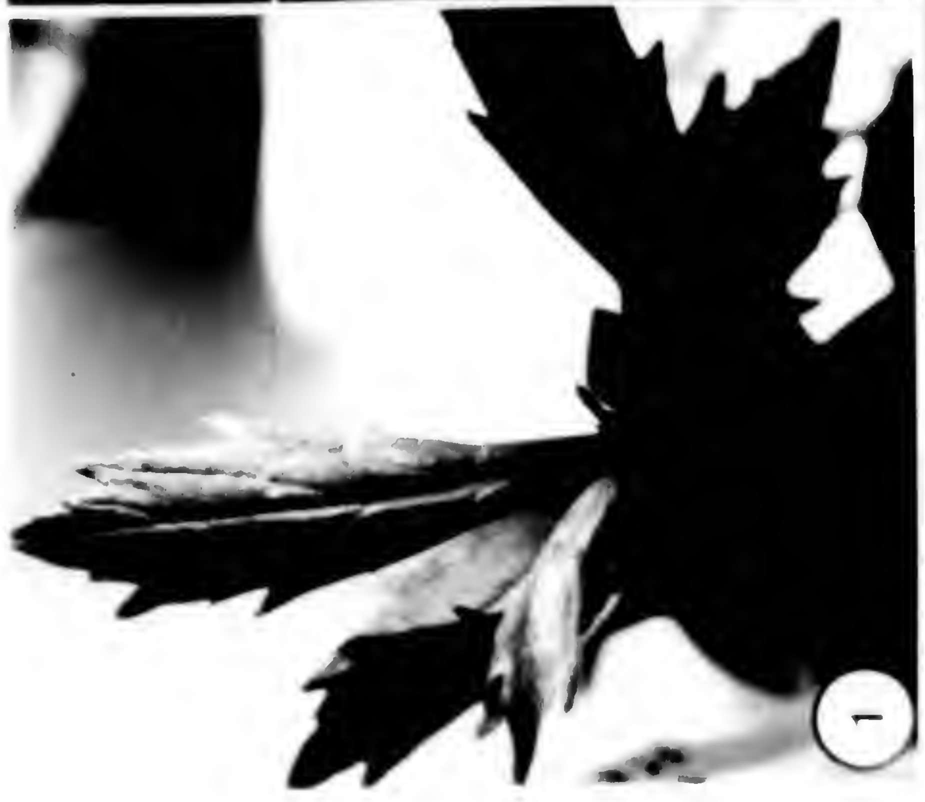
the Alaskan population showed a lower percentage of leaves injured in the early phase of the experiment for plants treated with pH 2.5 "rain", and a lower percentage of leaves injured during the entire experiment for plants treated with pH 3.0 "rain" (Fig. 4, Appendix 3). When the sensitivities of the individual clones from the two populations are examined, it can be noted that Smoking Hills clones overlap in their sensitivity with Alaskan clones. Clones 8 and 9 from the Smoking Hills had the highest percentages of leaves injured of the six clones, however clone 11 from the Smoking Hills fell within the range for the three Alaskan clones in the percentage of leaves which were injured (Figs. 7 and 8, Appendix 3). Results for the percent leaf area injured for the two populations also show an overlap in the sensitivities of clones from the two populations (Table 20).

There is no evidence to suggest that the Smoking Hills population is more tolerant of acid rain as a result of selection in an environment of extreme acidity. Clones of the two populations overlapped in their sensitivities to foliar injury, with Alaskan clones showing less injury overall. Both showed a high tolerance.

5. Differential sensitivities of foliage of *Artemisia tilesii*, *Phaseolus vulgaris* and *Spinacea oleracea* to simulated acid rain.

An experiment was performed to show the responses of foliage of *Artemisia tilesii*, *Phaseolus vulgaris* (wax bean) and *Spinacea oleracea* (spinach) to simulated acid rain. The objective was to determine the tolerance of *Artemisia tilesii* foliage to acid rain relative to other species. Two clones of *Artemisia*, clone 11 from the Smoking Hills and clone 3 from Alaska, were used for the comparison.

The results for Duncan's Multiple Range tests for the percent leaf area injured after 4 exposures at pH 2.5 and 3.0 (Table 21) show that spinach foliage had significantly more injury than the other species tested. About 20% of the leaf surface of leaves of spinach was injured after exposure to four rainfalls at pH 2.5 (two 20-min. rainfalls weekly). Leaves of some spinach plants had as much as 40% of the leaf surface area injured after exposure to four rainfalls at pH 2.5 (Table 22). Foliage of one clone of *Artemisia* exhibited less than 2% leaf area injury, while the other clone of *Artemisia*, and bean both had about 5% of the leaf surface area injured, after four rainfall exposures of pH 2.5 (Plates 1 - 3). At pH 3.0 all of the species were injured less than at pH 2.5. However, the same order of sensitivity to foliar injury was observed amongst the species tested (Table 21). The percentage of leaves which were injured for the plants after 1, 2, 3 and 4 exposures at pH 2.5 and 3.0 also shows spinach as having the highest sensitivity, followed by bean and the two *Artemisia* clones, in that order (Table 22, Fig. 9). Large differences in the morphology of the species tested and in the number of leaves produced on each species, makes this measure a less reliable indicator of species sensitivity than the percentage of leaf area injured. For this reason the magnitude of the differences in the percentage of injured leaves between the species may not be very meaningful, and only the general trends of highest to lowest sensitivity have been considered.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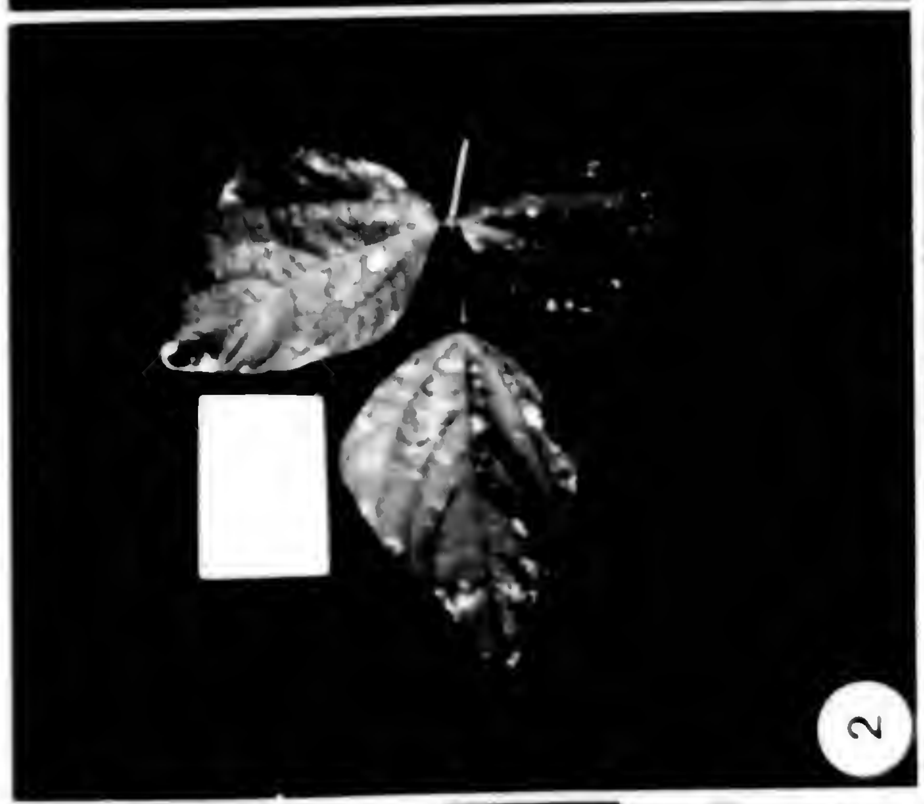
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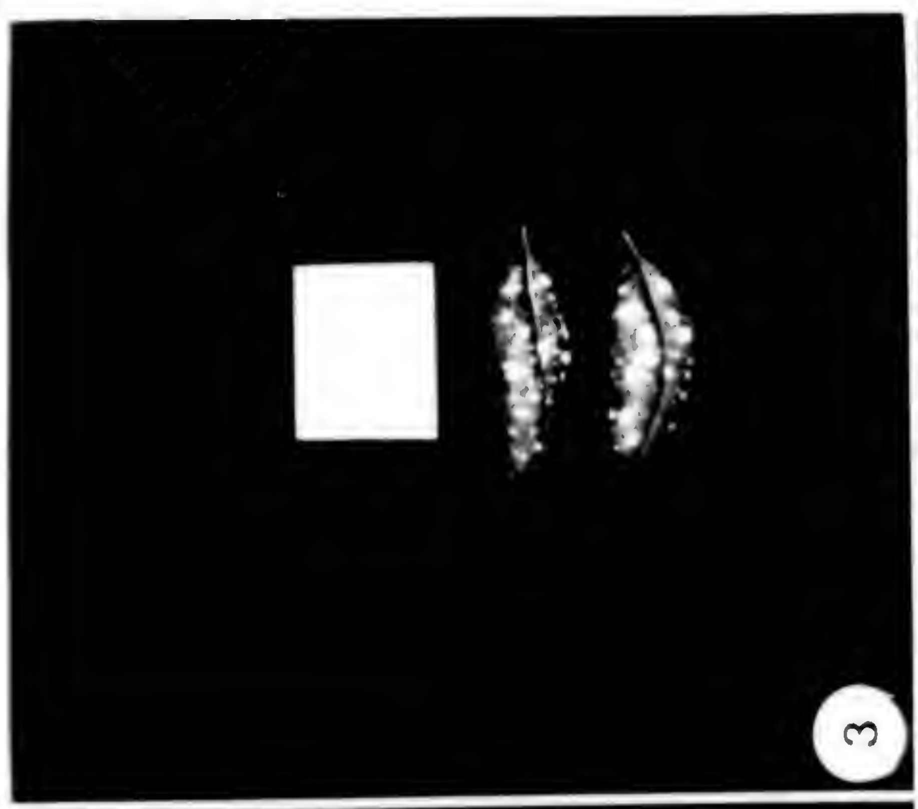
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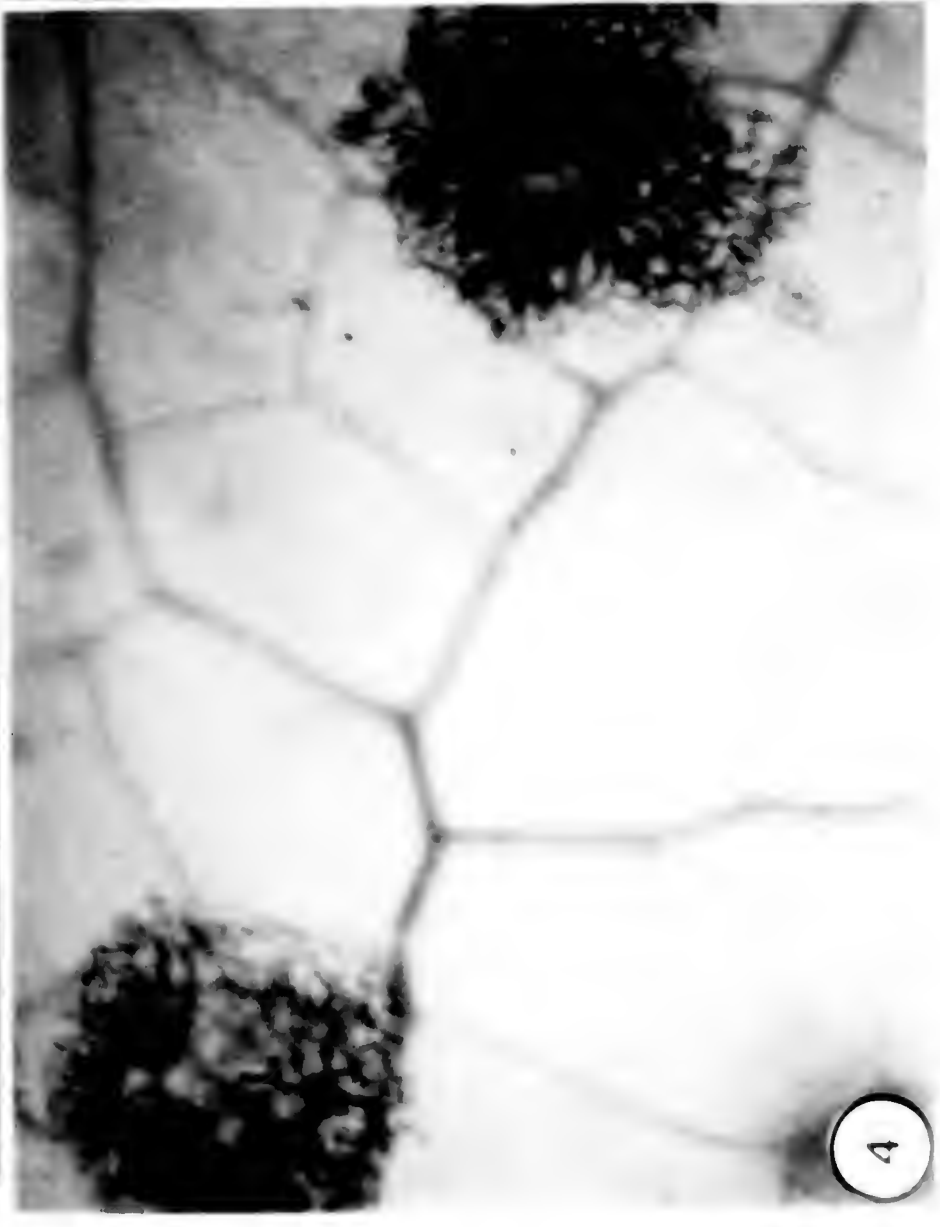
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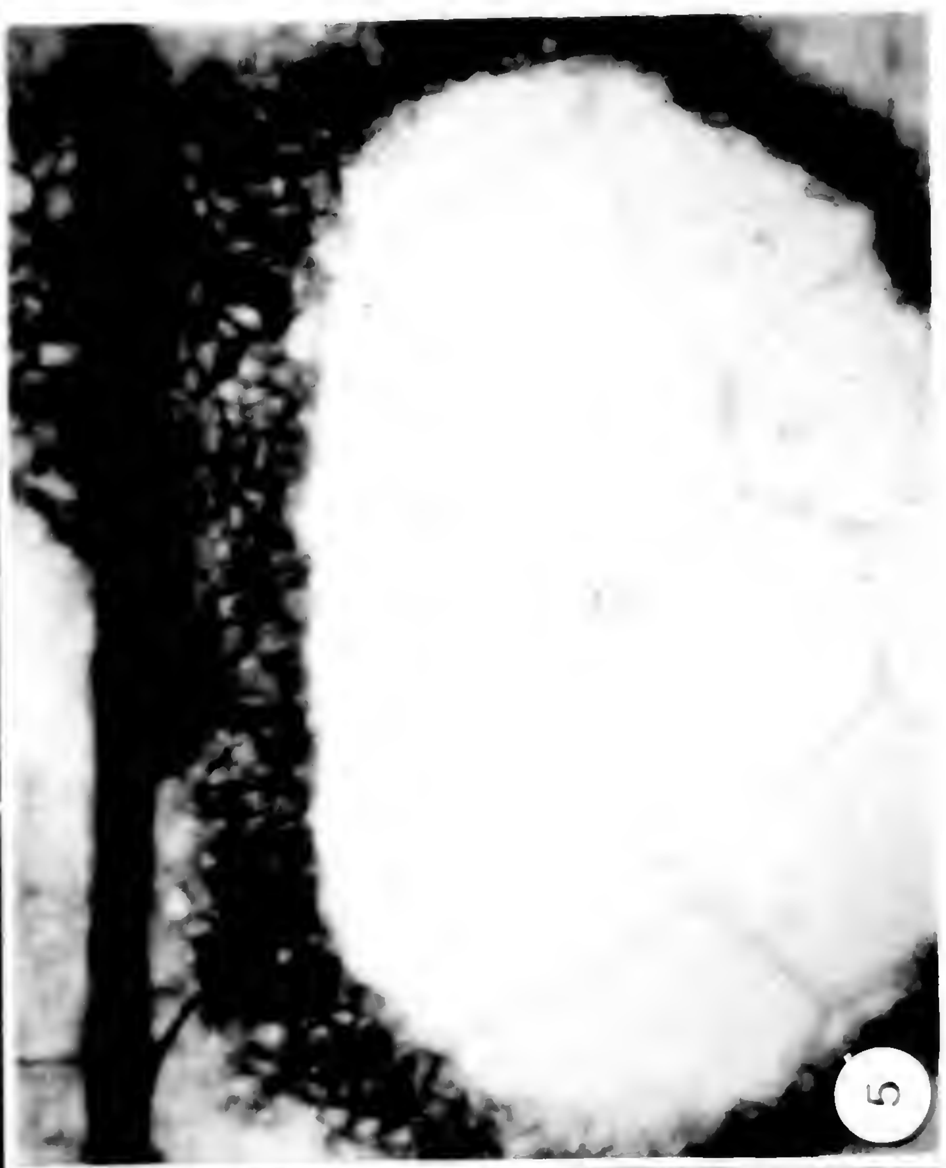
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Plates 1 - 3. Photographs of lesions on leaves of *Artemisia tilesii* (Plate 1), *Phaseolus vulgaris* (Plate 2) and *Spinacea oleracea* (Plate 3) after 4 twenty minute exposures to pH 2.5. Note extreme sensitivity of *Spinacea* leaves to injury in comparison with *Artemisia tilesii* and *Phaseolus vulgaris*.

Plates 4 and 5. Cleared leaves of *Artemisia tilesii*. Injured cells stain heavily with safranin making the outline of lesions (L) easily discernible. Plate 4. Two typical lesions which are circular in shape due to droplets resting on the leaf surface (X 58). Plate 5. A much larger lesion represented by a circular band of injured cells (I) on the leaf surface with no injury to cells interior to the band (X 54).

Table 21. Duncan's Multiple Range tests for the percent leaf area injured after 4 exposures (pH 2.5, 3.0) for *Artemisia tilesii*, *Phaseolus vulgaris* and *Spinacea oleracea*.

(Values are means of 10 plants ± 1 S.E.)
(Means with the same letter are not significantly different, $\alpha = 0.05$)

pH 2.5

	Spinacea	Phaseolus	Artemisia (Alaska 3)	Artemisia (Smoking Hills 11)
$\bar{x}$ % leaf area injured	A 20.5 ± 3.0	B 5.0 ± 1.5	B 5.5 ± 0.8	B 1.5 ± 0.4

pH 3.0

	Spinacea	Phaseolus	Artemisia (Alaska 3)	Artemisia (Smoking Hills 11)
$\bar{x}$ % leaf area injured	A 2.5 ± 0.0	B 1.5 ± 0.4	B 1.5 ± 0.4	C 0.0 ± 0.0

Plants exposed to pH 3.5, 4.0 and 5.6 showed no injury.

Table 22. The effect of pH on foliar injury of *Artemisia tilesii*, *Phaseolus vulgaris* (bean) and *Spinacea oleracea* (spinach).
(Values are means for 10 plants ± S.D.)[a]

	1 Spray		2 Sprays		3 Sprays		4 Sprays	
	% leaves injured	%leaf area injured	%leaves injured	%leaf area injured	% leaves injured	%leaf area injured	% leaves injured	%leaf area injured
pH 2.5								
Spinach	95.4 ± 6.1	5.0 ± 4.8 (max 17.5)	97.3 ± 4.4	7.0 ± 4.4 max 17.5)	99.1 ± 2.9	13.5 ± 5.2 (max 17.5)	100.0 ± 0.0	20.5 ± 9.5 (max 37.5)
Bean	94.2 ±12.3	2.5 ± 0.0 (max 2.5)	96.3 ± 7.8	3.0 ± 1.6 (max 7.5)	95.7 ± 4.1	3.0 ± 1.6 (max 7.5)	100.0 ± 0.0	5.0 ± 4.9 (max 17.5)
Artemisia (Alaska 3)	58.6 ±13.3	2.5 ± 0.0 (max 2.5)	75.5 ±16.8	2.5 ± 0.0 (max 2.5)	89.0 ±11.3	4.0 ± 2.4 (max 7.5)	94.2 ±10.0	5.5 ± 2.6 (max 7.5)
Artemisia (Smoking Hills 11)	13.7 ± 8.7	1.0 ± 1.3 (max 2.5)	23.0 ±10.8	1.9 ± 1.1 (max 2.5)	33.5 ±10.3	2.0 ± 1.0 (max 2.5)	44.2 ±16.4	1.5 ± 1.3 (max 2.5)

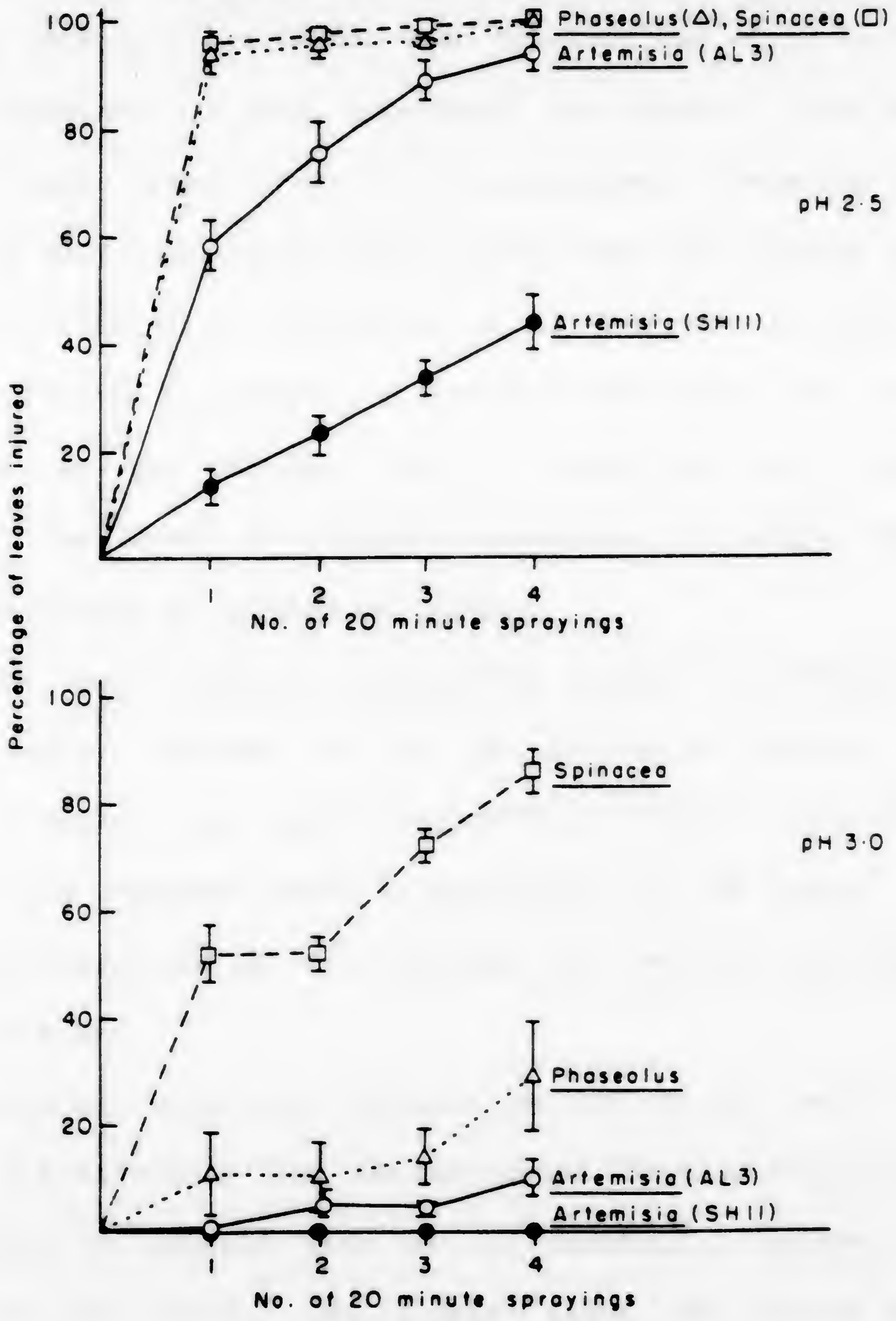
Continued

Table 22 (Continued).

	1 Spray		2 Sprays		3 Sprays		4 Sprays	
	% leaves injured	%leaf area injured	% leaves injured	%leaf area injured	% leaves injured	%leaf area injured	% leaves injured	%leaf area injured
pH 3.0								
Spinach	51.9 ±16.1	2.0 ± 1.0 (max 2.5)	52.1 ± 9.8	2.5 ± 0.0 (max 2.5)	72.4 ± 9.7	2.5 ± 0.0 (max 2.5)	86.2 ±13.0	2.5 ± 0.0 (max 2.5)
Bean	10.8 ±24.8	0.0 ± 0.0 (max 0.0)	10.5 ±19.8	0.0 ± 0.0 (max 0.0)	14.0 ±16.4	0.5 ± 1.0 (max 2.5)	28.6 ±30.2	1.5 ± 1.3 (max 2.5)
Artemisia (Alaska 3)	1.0 ± 2.3	0.2 ± 0.8 (max 2.5)	5.4 ± 7.5	0.5 ± 1.0 (max 2.5)	4.6 ± 4.6	1.0 ± 1.3 (max 2.5)	9.9 ±10.5	1.5 ± 1.3 (max 2.5)
Artemisia (Smoking Hills 11)	0.0 ± 0.0	0.0 ± 0.0 (max 0.0)	0.0 ± 0.0	0.0 ± 0.0 (max 0.0)	0.0 ± 0.0	0.0 ± 0.0 (max 0.0)	0.0 ± 0.0	0.0 ± 0.0 (max 0.0)

[a] Percent leaf area injured was scored on fully expanded leaves using 10 categories: 0%, 1-5%, 6-10%, 11-25%, 26-50%, 51-75%, 76-90%, 91-95%, 96-99%, 100%.
Plants exposed to pH 3.5, 4.0 and 5.6 showed no injury.

Figure 9 Comparison of the sensitivity of *Artemisia tilesii*, *Phaseolus vulgaris* and *Spinacea oleracea* to simulated acid rain.



B. Microscopic effects

1. Cellular injury as measured from cleared leaves of *Artemisia tilesii*

Leaf clearings were made of leaves of *Artemisia* at the end of four weeks of rainfall exposure (12 six min. sprayings) for the experiment in which 6 Smoking Hills clones were treated, and after 1, 2, 4, and 8 rainfall exposures (20 min. sprayings) for clone 11 from the Smoking Hills and clone 3 from Alaska for the experiment comparing clones from the Smoking Hills population with clones from the Alaskan population. Leaves were cleared to determine (a) if microscopic (cellular) leaf injury occurred in the absence of visible leaf injury (b) the extent of leaf injury at the cellular level in comparison with visible leaf injury (c) the effect of repeated exposures to acidic rain on the size and frequency of lesions on leaves.

Injured cells stained considerably darker in safranin than did uninjured cells. Because of this the outline of lesions, which were typically circular, was easily discernible (Plates 4 and 5) and their diameters were measured under a microscope at low power. Leaf area injured was calculated as r^2, assuming that lesions were approximately circular in shape.

No cellular injury was observed for any of the leaves exposed to pH 4.0 or 5.6 rainfall. For the two clones (Smoking Hills clone 11 and Alaska clone 3) treated with pH 3.5 rainfall, injured cells were observed in both cases. The Alaskan clone had injured cells after exposure to as few as two rainfalls (20 min. exposure, twice weekly), while eight applications were necessary for cellular damage to the Smoking Hills clone (Tables 23 and 24). While microscopic injury was present, no visible injury was observed on any of the 84 Artemisia plants treated with pH 3.5 rainfall even after exposure to as many as 24 rainfall episodes (Tables 23 and 24).

The extent of cellular injury increased greatly with decreasing pH (Tables 23 - 25). Because the microscopic measurements were confined to fairly small areas of cleared leaf tissue, this unfortunately does not allow comparison of the extent of cellular injury of individual clones. However, when microscopic leaf injury is compared with macroscopic leaf injury, the percent leaf area injured is consistently higher at the cellular level (occasionally by as much as 10X), than visual estimations of leaf surface injury (Tables 23 - 25). These results suggest that visual estimates of leaf surface injury may significantly underestimate the damage to leaves from acid rain.

In order to examine the size of lesions on injured leaf surfaces, lesions were grouped by their diameters as: less than 0.25 mm, 0.25 - 1.00 mm, 1.00 - 2.00 mm and greater than 2.00 mm. The highest percentage of lesions occurred in the smallest diameter size range (< 0.25 mm), equivalent to about five cells in diameter, with progressively fewer lesions in the larger size classes (Table 26). However, as the acidity of the rainfall increased higher percentages of large lesions occurred. At pH 2.0, 20% of all lesions were 1.00 mm or larger in diameter, compared to only 5% at pH 2.5 or 3.0. The total number of lesions on injured leaf surfaces also increased with the acidity of the rainfall treatment. In an area 185 mm^2, 311 lesions were produced by rainfall with a pH of 2.0, 192 with a pH of 2.5, and 55 with a pH of 3.0 (Table 26).

A similar shift towards larger sized lesions occurred if leaves were exposed to repeated treatment of acidic rainfall, at pH 3.0, 2.5, and 2.0 (Tables 23 and 24). The total number of lesions on leaves also increased as the number of 20 minute exposures to acidic rainfall increased from one to eight (Table 27). These results indicate that the increase in foliar injury caused by repeated exposure to simulated acid rain is caused by a combination of two factors: (a) the production of new lesions and (b) the enlargement of lesions already present.

Table 23. Response of an Alaskan clone (Al 3) after repeated exposure to simulated rain.

pH of simulated rain	Number of 20-minute exposures	Microscopic leaf injury (area of leaf occupied by lesions of various diameters[a])					Macroscopic leaf injury[b]	
		<0.25mm (um^2)	0.25-1.00mm (um^2)	1.00-2.00mm (um^2)	>2.00mm (um^2)	Total area injured (%)	Total leaf area injured (%)	Leaves injured (%)
2.5	1	96	0	0	0	<0.1	--	11
	2	--	--	--	-	--	--	15
	4	6,524	2,158	10,066	0	2.5	--	91
	8	3,289	730	14,437	0	2.5	2.3	90
3.0	1	149	0	0	0	<0.1	--	0
	2	3,971	1,393	0	0	<0.1	--	0
	4	4,524	2,085	0	0	<0.1	--	0
	8	8,085	34,842	0	0	5.8	0.0	1
3.5	1	0	0	0	0	0.0	--	0
	2	457	0	0	0	<0.1	--	0
	4	5,161	10,057	0	0	2.1	--	0
	8	2,490	2,429	0	0	<0.1	0.0	0
5.6	1	0	0	0	0	0.0	--	0
	2	0	0	0	0	0.0	--	0
	4	0	0	0	0	0.0	--	0
	8	0	0	0	0	0.0	0.0	0

[a] Each value represents the average from a minimum of ten areas (each .74mm^2) from cleared leaves of four plants.

[b] Each value represents the average of fourteen plants. Percent leaf area injured was assessed on one fully expanded leaf/plant.

Table 24. Response of a Smoking Hills clone (SH 11) after repeated exposure to simulated rain.

pH of simulated rain	Number of 20-minute exposures	Microscopic leaf injury (area of leaf occupied by lesions of various diameters[a])					Macroscopic leaf injury[b]	
		<0.25mm (um^2)	0.25-1.00mm (um^2)	1.00-2.00mm (um^2)	>2.00mm (um^2)	Total area injured (%)	Total leaf area injured (%)	Leaves injured (%)
2.5	1	0	0	0	0	0.0	--	17
	2	0	0	0	0	0.0	--	30
	4	437	0	0	0	<0.1	--	78
	8	4,608	64,762	70,777	0	19.0	3.6	85
3.0	1	21	0	0	0	<0.1	--	0
	2	0	0	0	0	0.0	--	0
	4	486	0	0	0	<0.1	--	0
	8	1,355	1,630	0	0	<0.1	0.5	3
3.5	1	0	0	0	0	0.0	--	0
	2	0	0	0	0	0.0	--	0
	4	0	0	0	0	0.0	--	0
	8	154	0	0	0	<0.1	0.0	0
5.6	1	0	0	0	0	0.0	--	0
	2	0	0	0	0	0.0	--	0
	4	0	0	0	0	0.0	--	0
	8	0	0	0	0	0.0	0.0	0

[a] Each value represents the average from a minimum of ten areas (each .74mm^2) from cleared leaves of four plants.

[b] Each value represents the average of fourteen plants. Percent leaf area injured was assessed on one fully expanded leaf/plant.

Table 25. Microscopic and macroscopic injury to foliage of Smoking Hills clones after exposure to simulated acid rain (12 six min. exposures).

Clone (Smoking Hills Pop'n)	Rainfall pH	Microscopic Injury[a]		Macroscopic Injury[b]	
		Total area injured (μm^2)	% Leaf area injured	% Leaf area injured	% Leaves injured
3	2.0	7,660,000	****[b]	33.5	100
	2.5	10,300,000	****	5.2	98
	3.0	150,000	2.4	2.3	80
5	2.0	14,100,000	****	54.8	100
	2.5	585,000	9.5	6.0	95
	3.0	938,000	15.2	3.1	55
8	2.0	12,500,000	****	58.1	100
	2.5	2,550,000	41.4	13.7	92
	3.0	366,000	5.9	2.7	57
9	2.0	6,540,000	****	28.3	100
	2.5	389,000	6.3	3.5	97
	3.0	147,000	2.4	2.3	83
11	2.0	18,900,000	****	53.1	99
	2.5	1,130,000	18.3	7.4	84
	3.0	194,000	3.1	2.3	24
12	2.0	8,990,000	****	46.3	97
	2.5	2,120,000	34.4	3.5	87
	3.0	768,000	12.5	2.2	36

[a] Values are the mean of 5 plants. Four areas for each cleared leaf (giving a total area of 6.16 mm^2) were examined.

[b] Percent leaf area injured values for pH 2.0 cannot be calculated since measured lesions were often larger than the field of measurement (6.16 mm^2).

[c] Values are the mean of 10 plants. Three leaves were used to determine % leaf area injured for each plant.

NOTE: No macroscopic or microscopic injury occurred on leaves of plants treated with rainfall at pH 4.0 or 5.6.

Table 26. Percent of lesions in four size classes and total number of lesions for Smoking Hills clones after exposure to simulated acid rain (12 six min. exposures).
(Values are derived from 5 plants for each of 6 clones)

Rainfall pH	Percent of lesions in four size categories (%)				Total number of lesions in an area of 185 mm^2
	<0.25mm diameter	0.25-1.00mm diameter	1.00-2.00mm diameter	>2.00mm diameter	
2.0	53	27	13	7	311
2.5	60	35	4	1	192
3.0	73	20	5	2	55

No cellular damage occurred from exposure to rainfall at pH 4.0 or 5.6.

Table 27. Mean number of lesions falling into each size class after repeated exposures to simulated rain (based on an area of 10 fields = 7.4 mm^2) (n = 4 plants).

Rain pH	Number of exposures	Mean number of lesions in each size range									
		<.25mm		.25 - 1.00mm		1.00 - 2.00mm		>2.00mm		Total	
		SH[a]	AL[b]	SH	AL	SH	AL	SH	AL	SH	AL
2.5	1	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
	2	0.00	-	0.00	-	0.00	-	0.00	-	0.00	-
	4	0.45	6.14	0.00	0.34	0.00	0.11	0.00	0.00	0.45	6.59
	8	2.94	1.74	1.97	0.12	0.47	0.12	0.00	0.00	5.38	1.98
3.0	1	0.09	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.18
	2	0.00	3.00	0.00	0.30	0.00	0.00	0.00	0.00	0.00	3.30
	4	0.31	3.11	0.00	0.30	0.00	0.00	0.00	0.00	0.31	3.41
	8	0.76	3.96	0.11	2.01	0.00	0.00	0.00	0.00	0.87	5.98
3.5	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	4	0.00	2.84	0.00	0.64	0.00	0.00	0.00	0.00	0.00	3.48
	8	0.14	1.38	0.00	0.22	0.00	0.00	0.00	0.00	0.14	1.60
5.6	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a] SH = Smoking Hills clone.
[b] AL = Alaska clone.

c. Scanning electron microscopy

Leaf surfaces of control and acid-injured leaves were examined using scanning electron microscopy. Control leaves all exhibited normal, turgid epidermal cells as seen in Plate 15 for Smoking Hills *Artemisia* and Plate 18 for Alaskan *Artemisia*. The obvious difference between the *Artemisia* leaves on plants from these two populations was the presence of numerous stomata on the adaxial leaf surface of the Smoking Hills material, which were lacking on the adaxial epidermis of Alaskan *Artemisia*.

SEM examination of acid-treated leaves (pH 3.0) provided evidence of microscopic injury to the leaf surfaces, despite the fact no macroscopic injury was exhibited on these particular leaves. Numerous small lesions, consisting of one or two collapsed epidermal cells, were observed on several leaves taken from Alaskan (clone 3) plants exposed to eight applications of rainfall having a pH of 3.0 (Plates 7 and 8). Leaves receiving fewer applications of the acidic rain were, unfortunately, not available for examination. Leaves treated with pH 2.5 exhibited significantly larger lesions, with both a greater area of leaf surface injured and a greater depth of injury (Plates 9 and 10). Plate 9 shows a large crater-like lesion, consisting of 15 - 20 collapsed epidermal cells, surrounded by normal turgid cells. Damage to internal tissues is probably correlated with depression depth. Plate 10 shows an extensive area of leaf surface injured by acidic rain (pH 2.5), where injury is clearly affecting more internal tissues at the near end of the lesion. This large lesion is probably the result of two smaller lesions coalescing after repeated exposures to simulated acid rain. A small band of uncollapsed cells traversing the lesion at about the midpoint is probably the remnants of healthy tissue which once separated the two lesions.

SEM examination revealed significant differences in stomatal aperture size on the adaxial surface (Smoking Hills), between plants treated with acidified rain solutions and those treated with rain of pH 5.6 (control). In Plate 11 an open stomate is shown among injured epidermal cells. At higher magnification (Plate 12) it is clear that another stomate bordering the injured cells is also open. Stomata situated in the uninjured epidermal cells surrounding the lesion are closed, as were all stomata on leaves from the control rainfall treatment (pH 5.6). Numerous open stomates seen in severely collapsed tissue in Plate 13 suggest acidified rain solutions may interfere, in some way, with the normal functioning of guard cells. Plate 14 shows one of these open stomata at a higher magnification.

d. Relationship of lesion development with leaf surface features

It has been suggested that leaf surface features may be important in influencing sensitivity to injury (Evans et al, 1977b). Experiments with a number of species showed injury occurred most frequently adjacent to vascular tissues, trichomes and stomata (Evans et al, 1978; Evans and Curry, 1979). In these studies many of the lesions associated with leaf surface features however, were of considerable size and no attempt was made to separate the percentage of lesions which developed initially at these leaf surface features and those that increased in diameter after several rainfalls so as to affect these features. Some lesions, after only a single rainfall were also very large and it might be surprising if they did not occur over or adjacent to leaf surface features, particularly vascular tissue which underlies a considerable amount of epidermal cell surface area.

In this study, measurements were undertaken of the percentages of lesions occurring near veins (vascular tissues) for both Smoking Hills and Alaskan Artemisia from cleared leaves (Table 28). 483 lesions were scored for one experiment, in which only Smoking Hills clones were used, and in the second experiment 52 and 241 lesions were scored respectively for Smoking Hills and Alaskan clones. The percentage of lesions localized near vascular tissues varied considerably depending on the size of the lesion (Table 28). 95 - 100% of lesions in the largest size class (0.25 - 1.00 mm in diameter) occurred over vascular tissue. A more accurate and conservative estimate of lesions which developed over or directly adjacent to vascular tissue is around 35 - 40%, for lesions <0.10 mm in diameter. Lesions of this size are comprised of one or two collapsed epidermal cells and thus give a true indication of where the lesion initially developed.

The average distance between vascular bundles (Table 29) and the average width of vascular bundles (Table 30) were used to determine the ratio of leaf surface overlying vascular tissue to that of leaf surface overlying mesophyll tissue. Using these ratios for the two clones it is possible to determine if 35 - 40% of lesions originating over or adjacent to vascular tissues is higher than what would be expected if lesions were distributed randomly on the leaf surface (i.e. with no association with leaf surface features such as veins). The calculations presented in Appendix 5 show a convincing positive association of lesion development with vascular tissues, though this was not an invariable association. 1.7 (Alaska) and 1.86 (Smoking

Hills) times the number of lesions occurred over mesophyll tissue as occurred over vascular tissue, while the expected ratio was 2.3 (Alaska) and 2.6 (Smoking Hills) times as many lesions over mesophyll tissue as over vascular tissue.

No attempt was made to determine percentages of lesions occurring near stomata, or near glandular trichomes (which were infrequent on the leaf surface). SEM examination showed some lesions occurring near glandular trichomes (Plates 9 and 10) and others which obviously developed some distance away (Plate 7).

Table 28. Localization of lesions over vascular tissues (veins)[a]

Lesion Diameter (µm)	Lesions occurring over or directly adjacent to vascular tissue	
	EXPERIMENT 1	EXPERIMENT 2
	(%)	(%)
< 100	SH 35 (n=109 lesions)	SH 50 (n=30 lesions) AL 37 (n=131 lesions)
< 250	SH 50	SH 69 AL 48
< 1000	SH 65	SH 77 AL 59
250 - 1000	SH 95	SH 100 AL 93

[a] Total number of lesions scored for experiment #1 was 483 and for experiment #2 was 52 and 241 respectively for Smoking Hills and Alaskan clones.

Table 29. Distance between vascular bundles as calculated from leaf cross sections.

	Average distance between vascular bundles (µm)			
	5.6	3.0	2.5	Overall $\bar{x}$
	$\bar{x}$ SE	$\bar{x}$ SE	$\bar{x}$ SE	$\bar{x}$ SE
Smoking Hills	310.8 ± 30.93	290.5 ± 31.6	238.1 ± 28.7	276.9 ± 17.8 (n=92)
Alaska	311.3 ± 31.7	219.3 ± 19.3	275.3 ± 33.5	264.3 ± 16.7 (n=89)

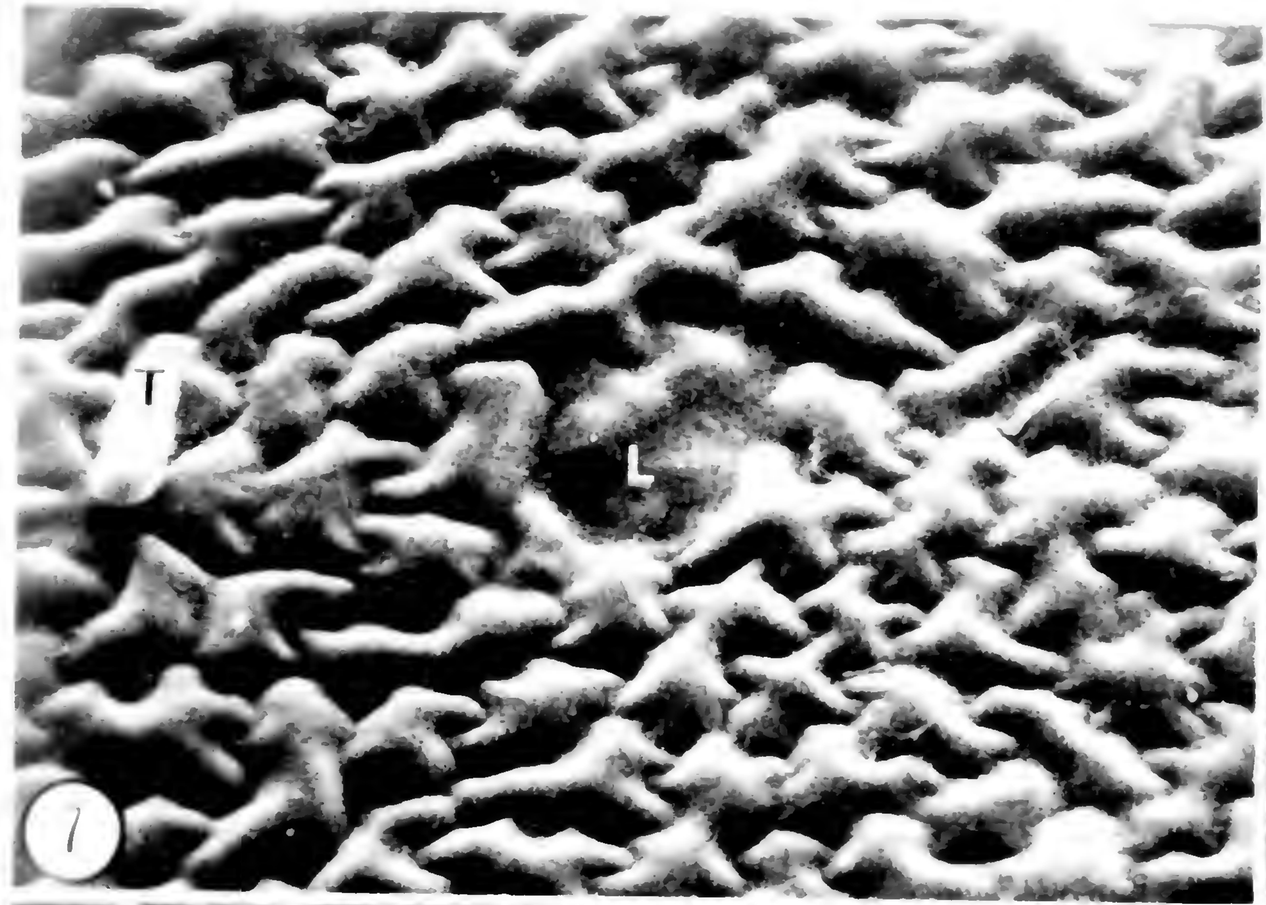
Table 30. Width of vascular bundles as calculated from leaf cross sections.

	Average width of vascular bundles (µm)	
	$\bar{x}$ ± SE	N
Smoking Hills	79.4 ± 3.5	33
Alaska	63.9 ± 2.8	31

Plates 7 - 10. Scanning electron micrographs of the adaxial leaf surface of Artemisia tilesii after exposure to simulated rain at pH 2.5.

Symbols: T = stalked capitáte trichome; L = lesion produced by simulated acid rain.

Plate 7. A small lesion with two collapsed epidermal cells (X 310). Plate 8. Same lesion as in Plate 10 (X 1000). Plate 9. Injured cells surround the remnants of two stalked capitáte trichomes (T). The circular lesion is bordered by uninjured epidermal cells (X 310). Plate 10. Low magnification (X 100) of a leaf surface that exhibits extensive damage. More internal cells are injured in the area in the foreground (arrow) as denoted by lesion depression depth.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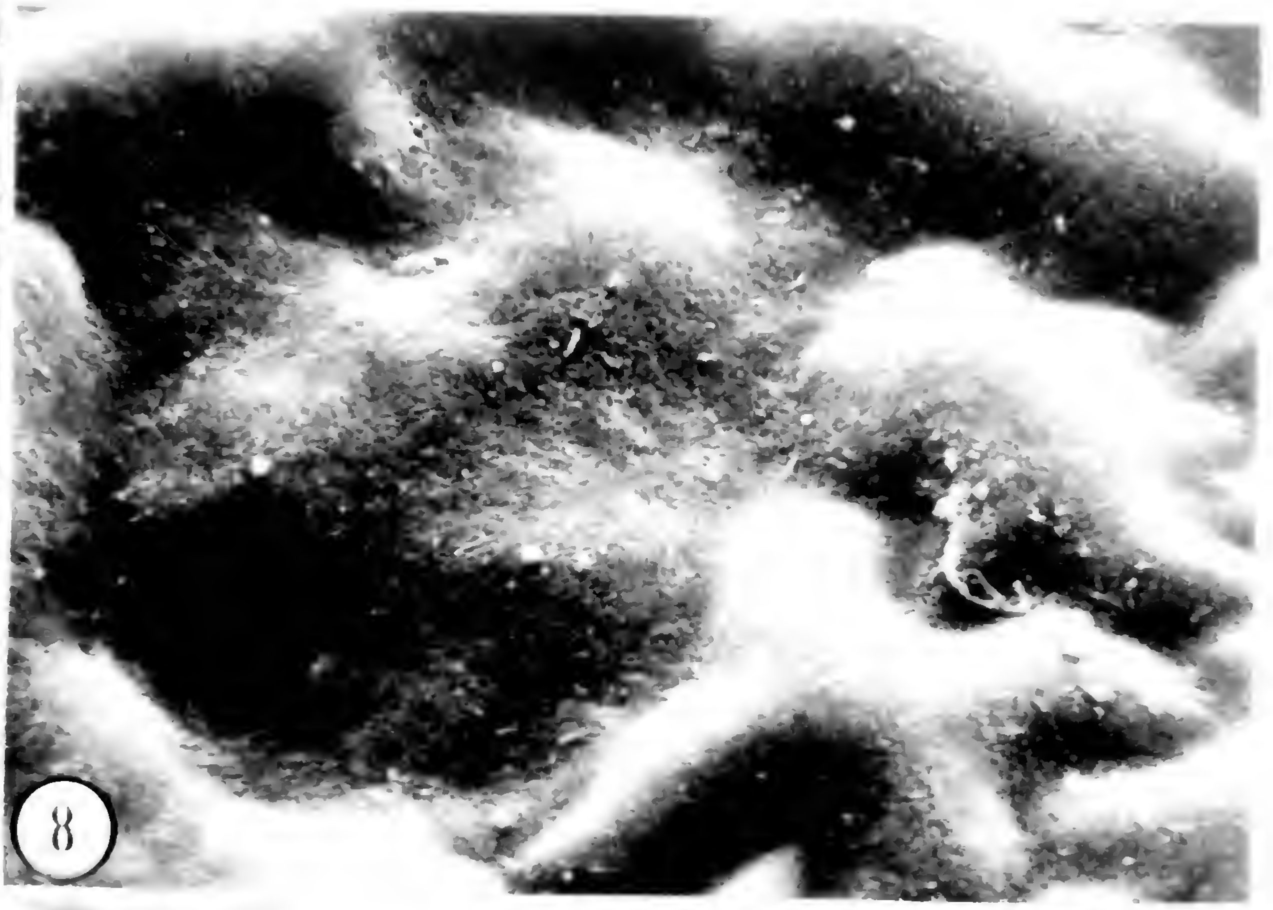
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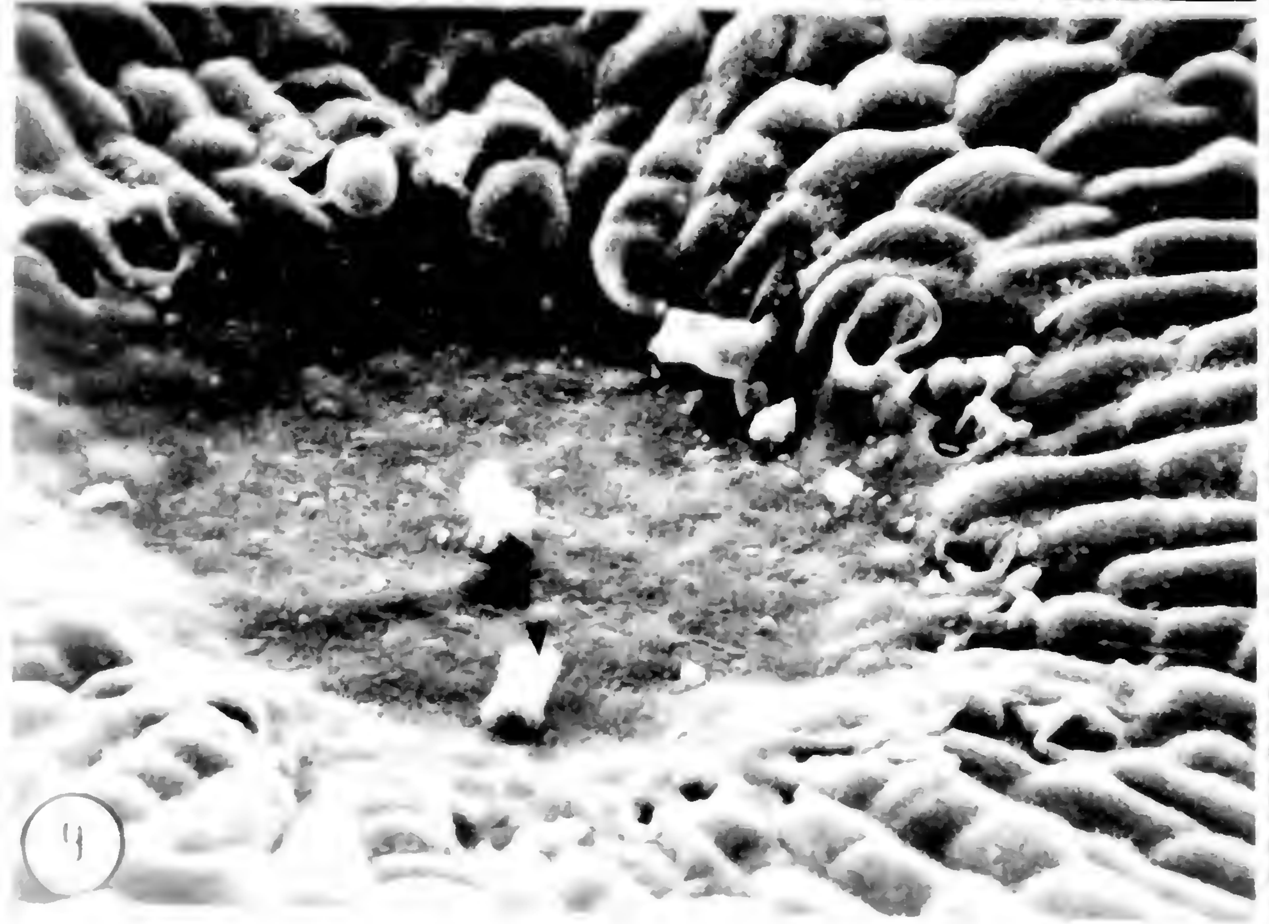
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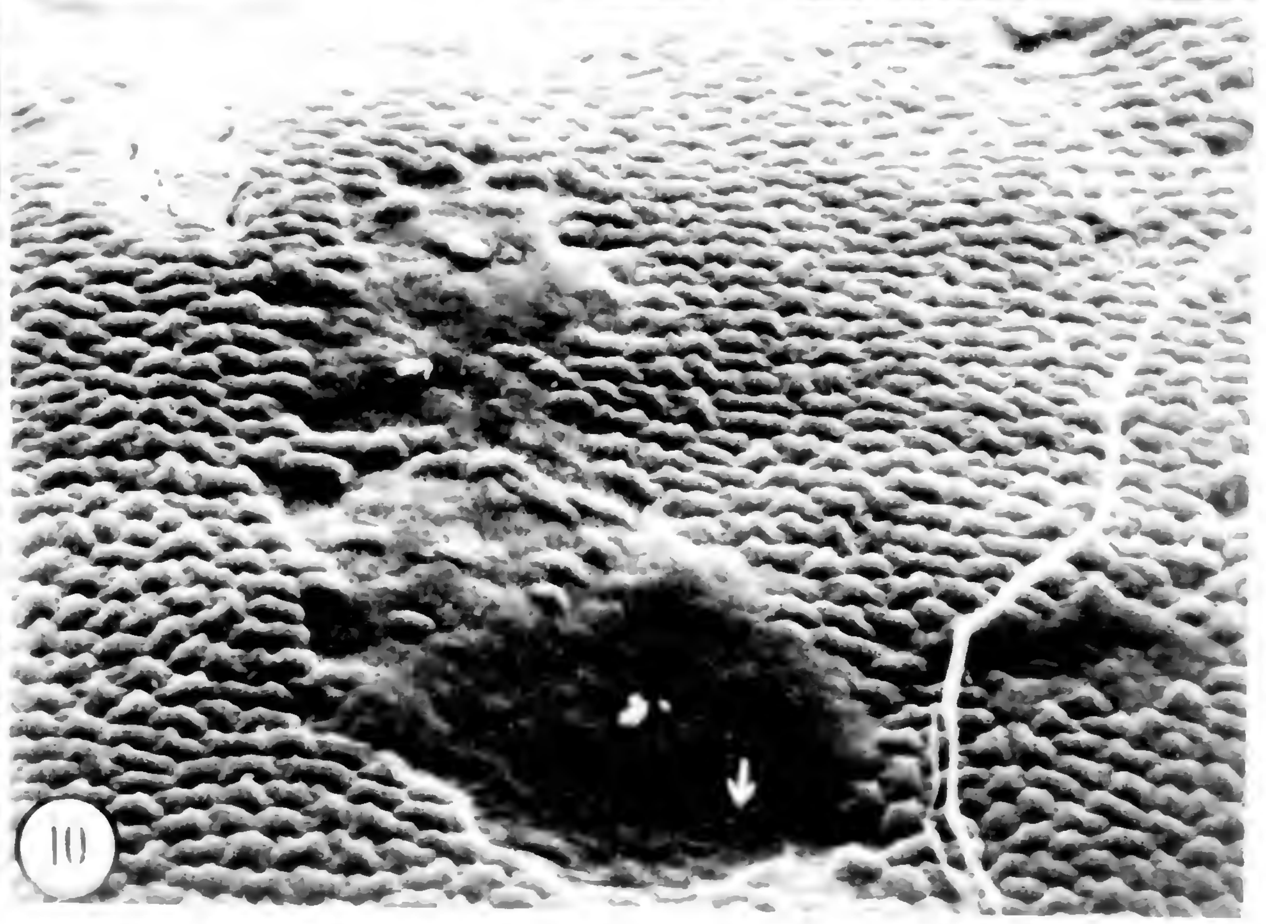
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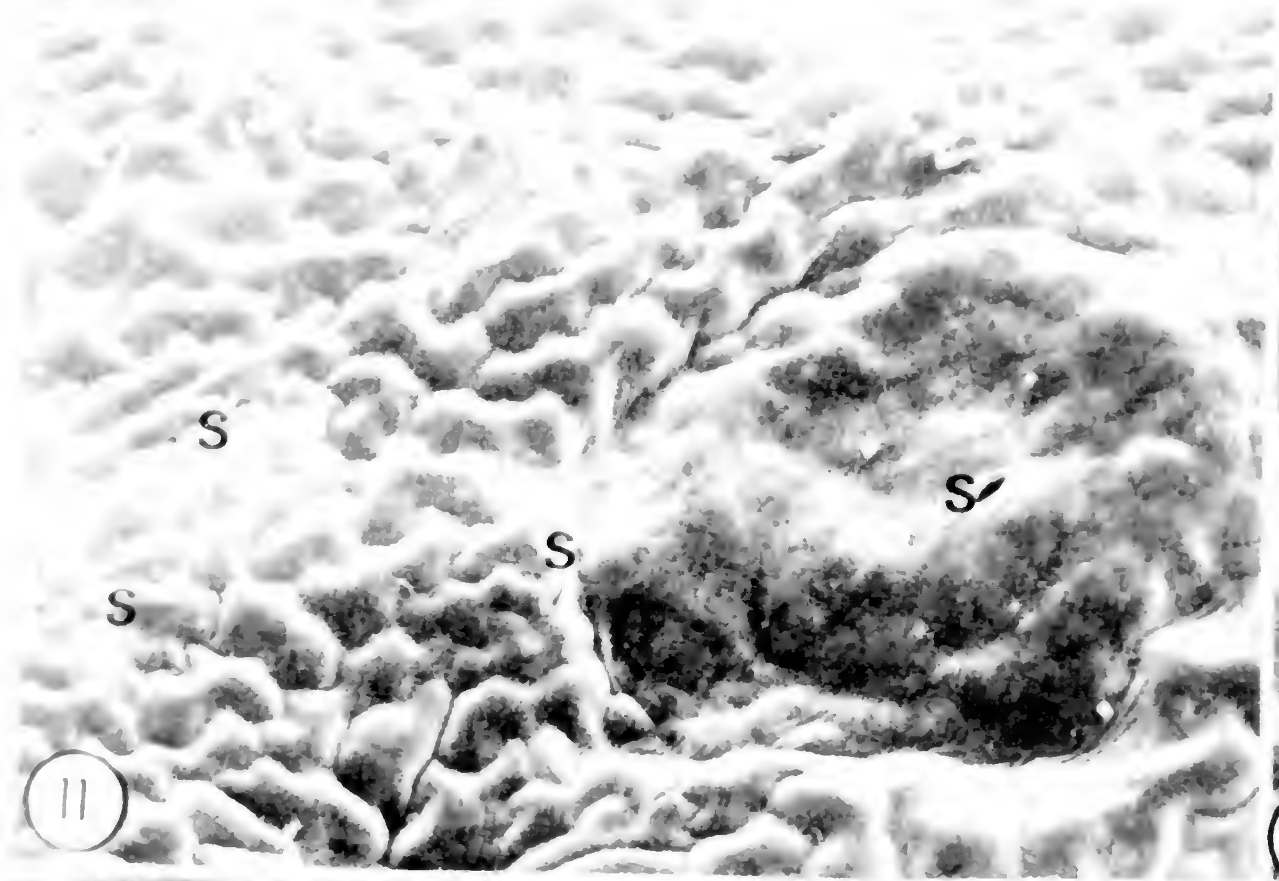
Plates 11 - 14. Scanning electron micrographs of the adaxial leaf surface of Smoking Hills *Artemisia tilesii* after exposure to simulated rain at pH 2.5. Plate 11. Large area of injured cells bordered by uninjured epidermal cells (X 160). Note stomate (S) in injured tissue is open while those in the healthy tissue adjacent are closed. Plate 12. Same lesion as in Plate 11 (X 365) illustrating the open passage into internal tissue created by the open stomate (S). Another stomate at the edge of the lesion is also open. Plate 13. Collapsed epidermal cells. Note stomata (S) in injured tissue are all open (X 317). Plate 14. Injured adaxial surface illustrating guard cells (G) which have collapsed leaving the stomate open (X 1000).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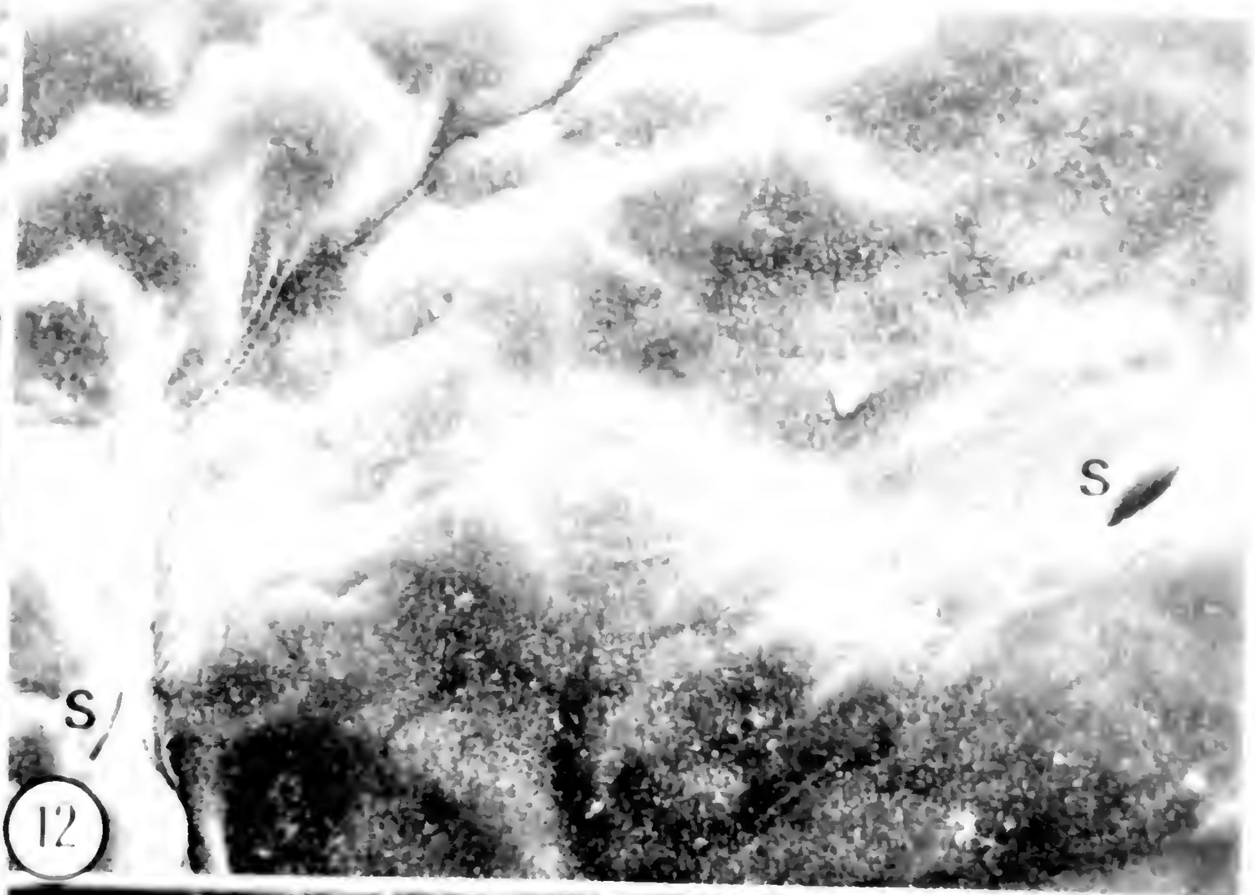
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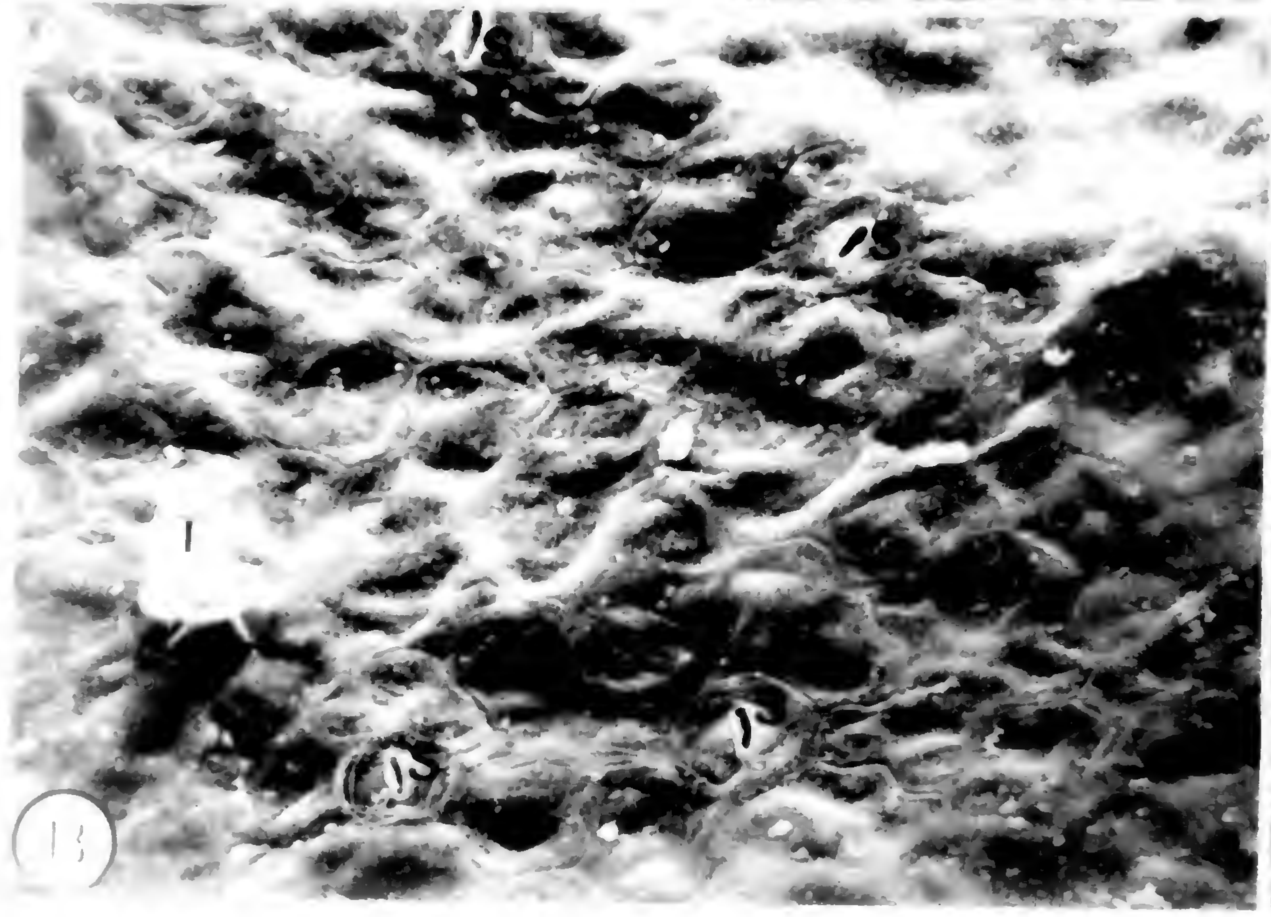
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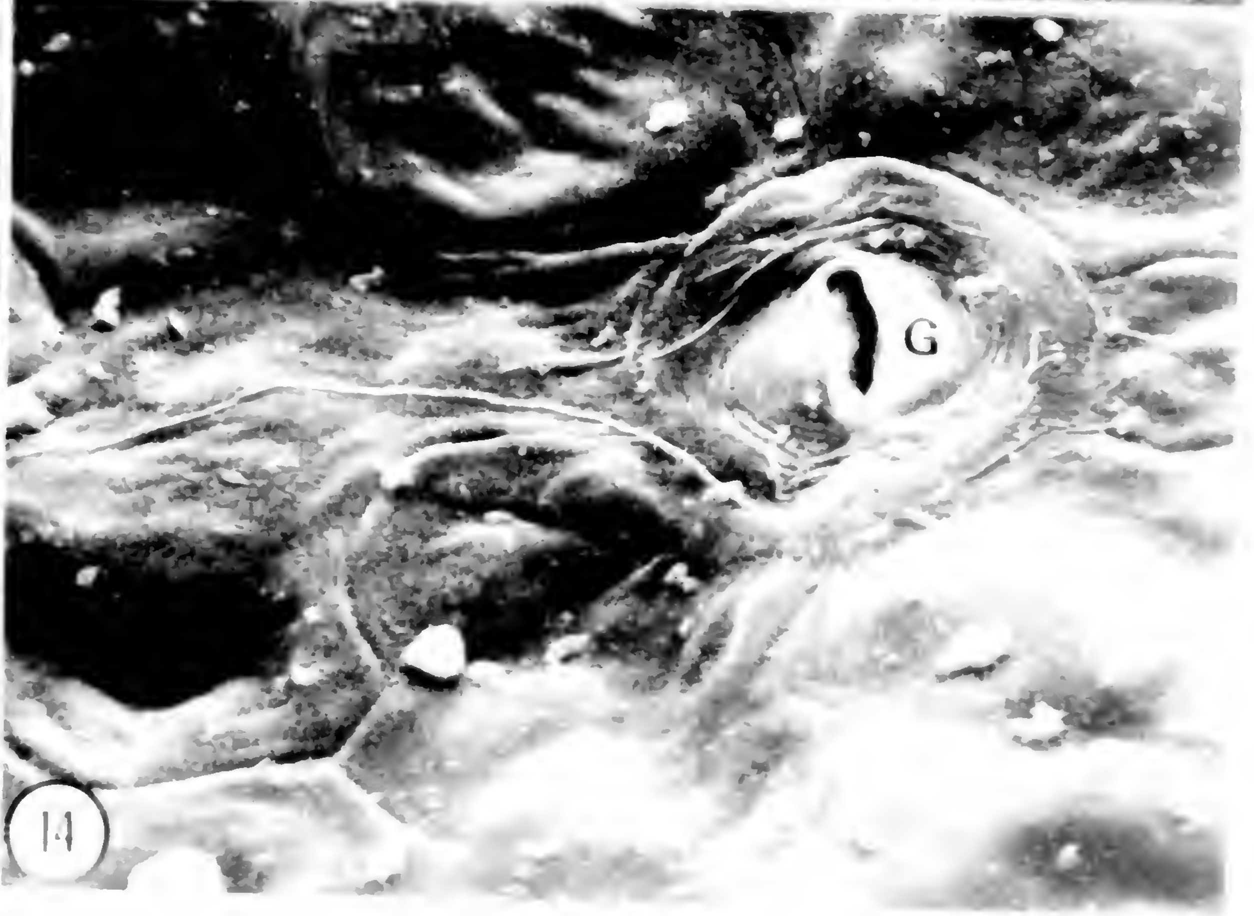
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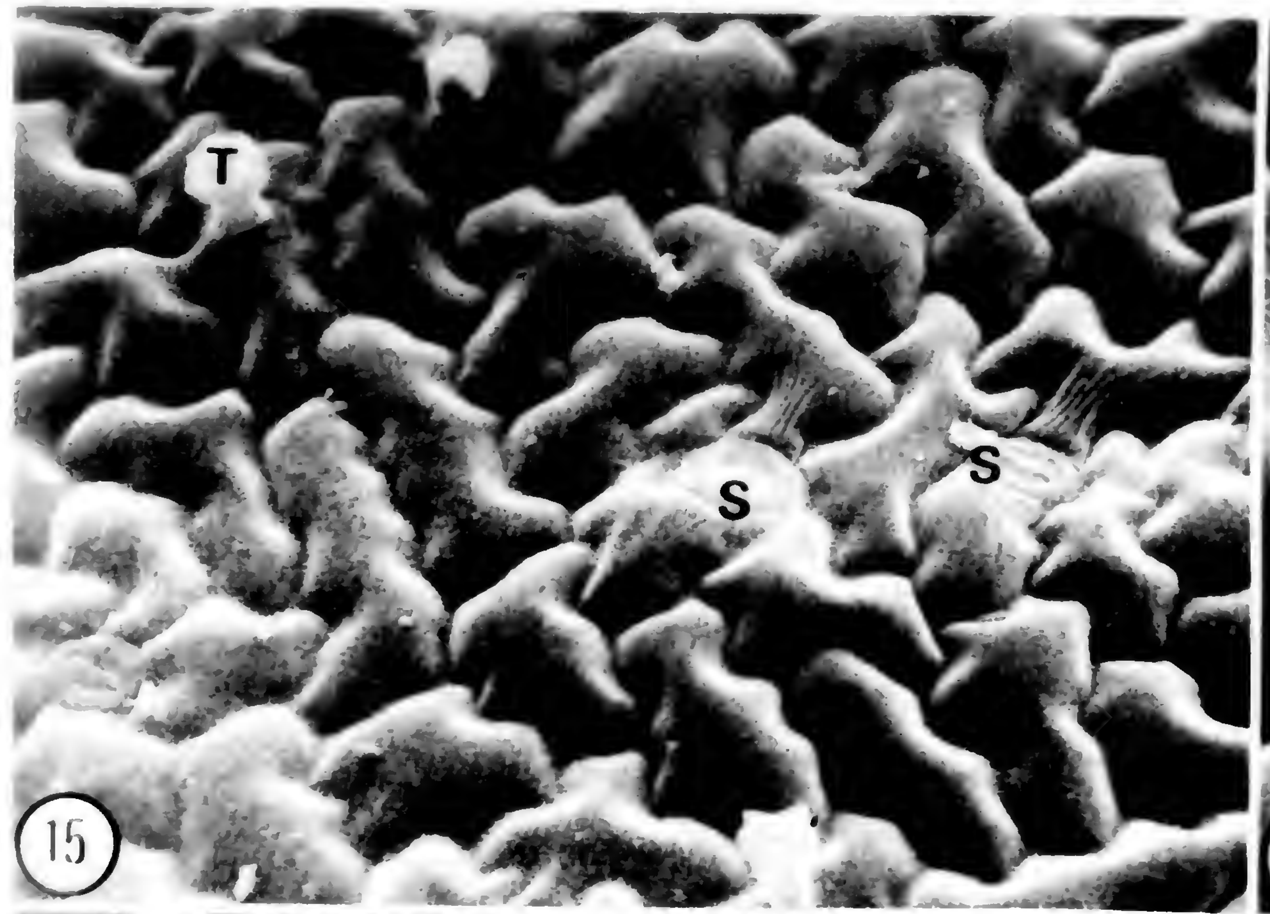
Plates 15 - 19. Scanning electron micrographs of healthy leaves of *Artemisia tilesii* to show the differences in leaf anatomy of plants from the Smoking Hills and Alaskan populations. Plate 15. Adaxial surface of a leaf from a Smoking Hills plant illustrating numerous stomata (S). Stalked capitáte trichomes (T) are also present on the adaxial epidermis (X 316). Plate 16. Same adaxial leaf surface from a Smoking Hills plant illustrating a stomate (X 1000). Note ridges on guard cells and on epidermal cells which surround the guard cells. Plate 17. Cross section of a leaf from a Smoking Hills plant (X 110). Note there is a greater number of cell layers than in leaves from Alaskan plants (Plate 19). Numerous multicellular trichomes (MT) form a dense mat on the abaxial surface of the leaf. Plate 18. Adaxial surface of a leaf from an Alaskan plant illustrating the absence of stomata (X 316). Plate 19. Cross section of a leaf from an Alaskan plant (X 316) illustrating fewer cell layers than leaves of Smoking Hills plants. Stalked capitâte trichomes occur on the adaxial epidermis (T) and multicellular trichomes (MT) cover the lower surface of leaves.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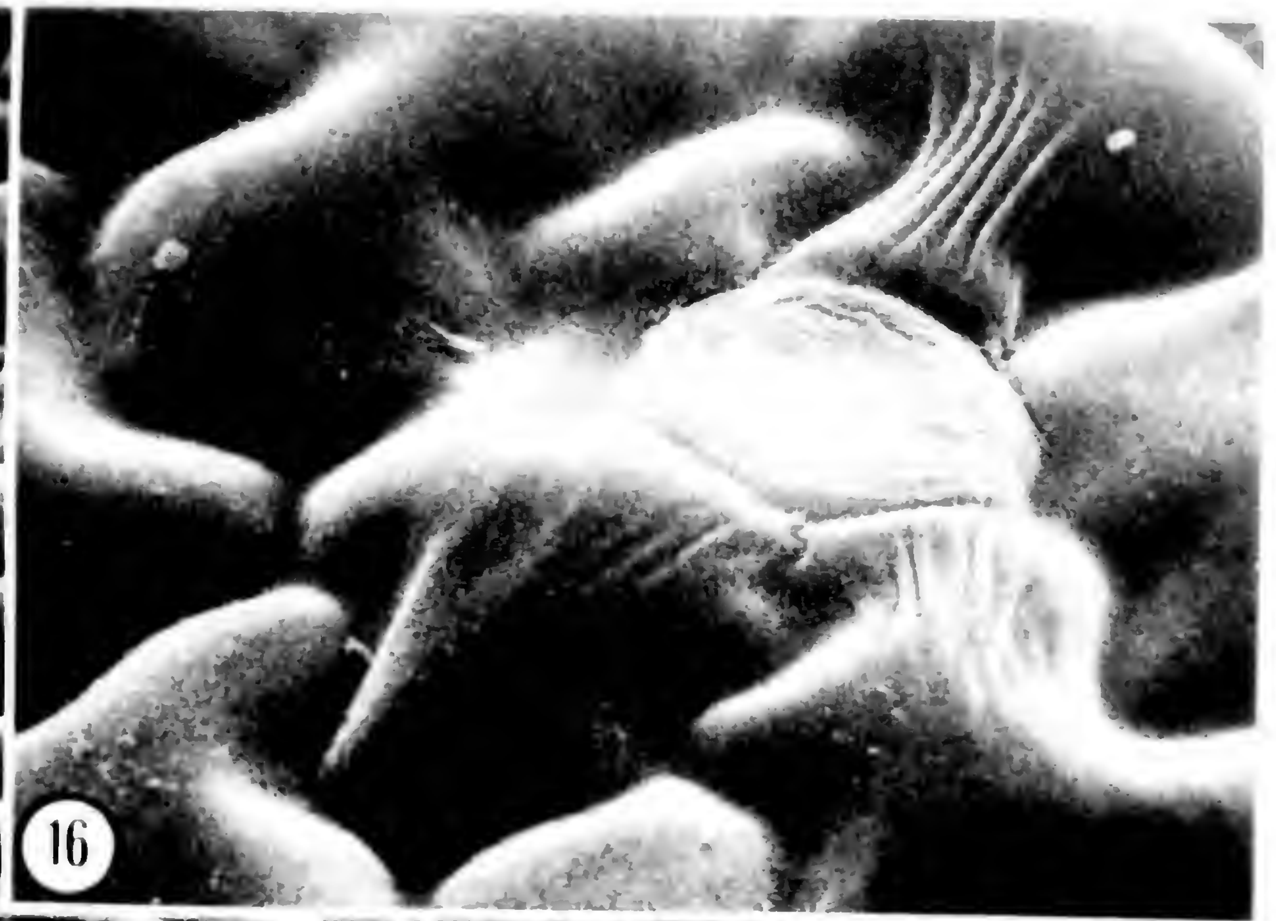
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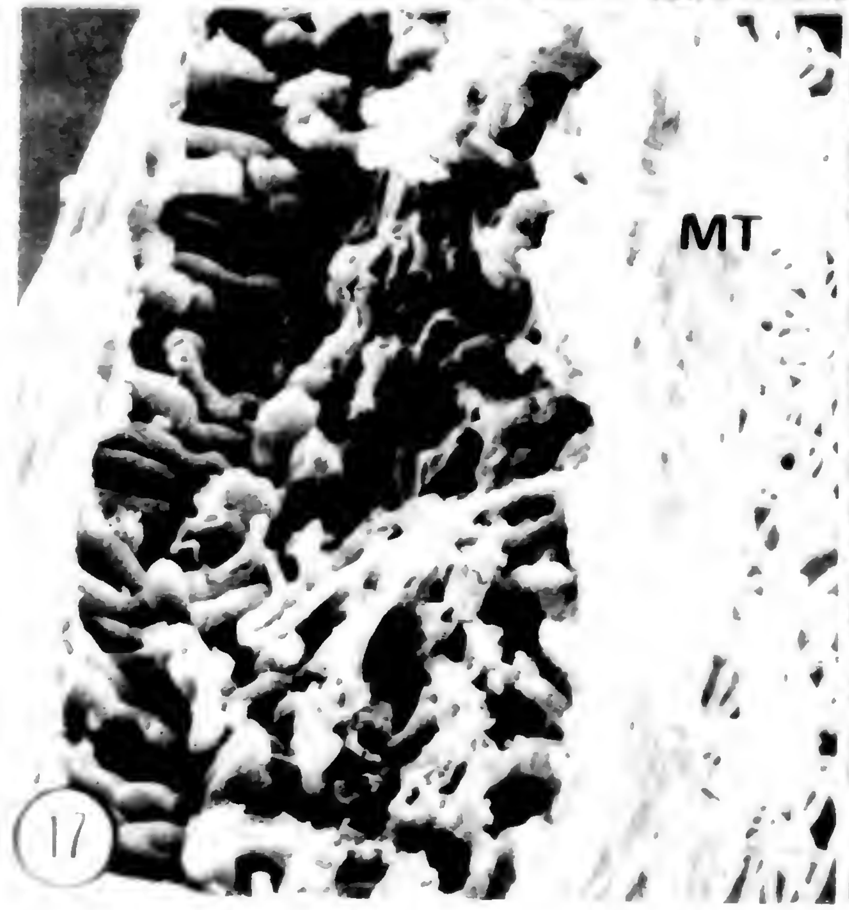
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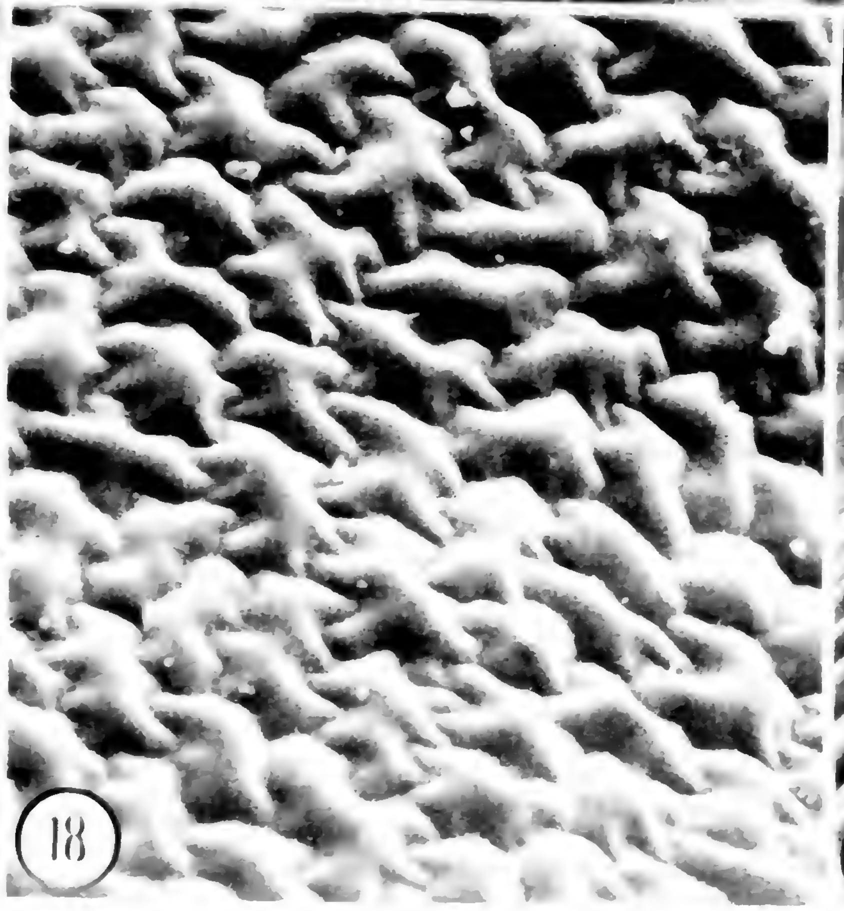
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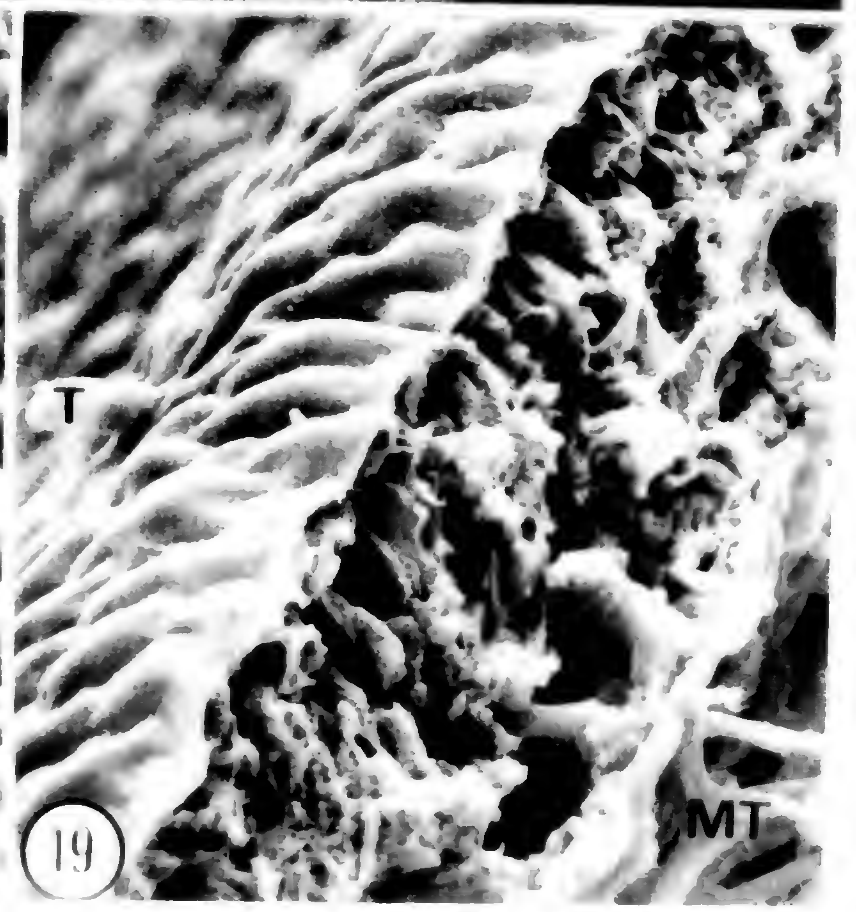
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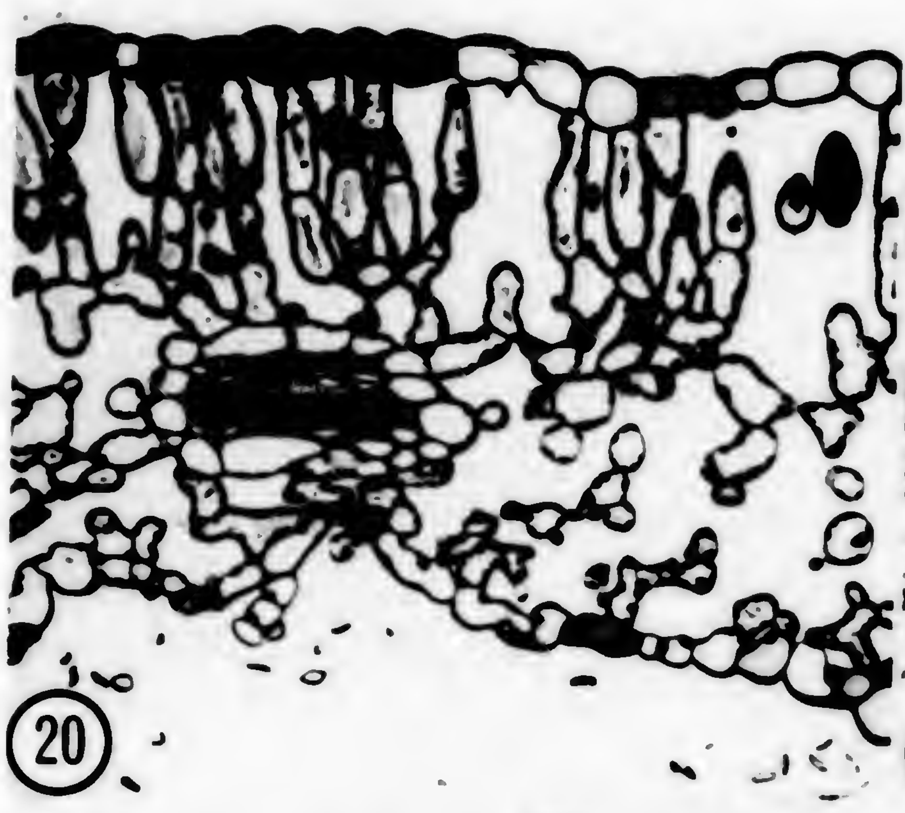
Plates 20 - 25. Leaf cross sections of Smoking Hills Artemisia tilesii, after one to several rainfalls of pH 5.6 (control rainfall) or pH 2.5 (acidic rainfall). Plates 20 and 21. Uninjured leaf tissue of a Smoking Hills clone after exposure to rainfall of pH 5.6 (X 133, X 167). Plates 22 - 24. The initial stage (Stage 1) in lesion development. Plate 22. Partial collapse of a small number of epidermal cells (CE) (X 314). Plate 23. Collapse of scattered epidermal cells (CE) interspersed with turgid epidermal cells. Note epidermal cells appear to collapse initially at the anticlinal walls producing cells which are flattened at each end almost to a point, giving them an oval shape. Uninjured cells are square to rectangular in shape with distinct cross-walls between cells (X 118). Plate 24. Initial injury of epidermal cells near a stalked capitâte trichome (T) (X 125). Plate 25. Two guard cells(G) have maintained their integrity in the adaxial surface while surrounding epidermal cells have collapsed (X 103).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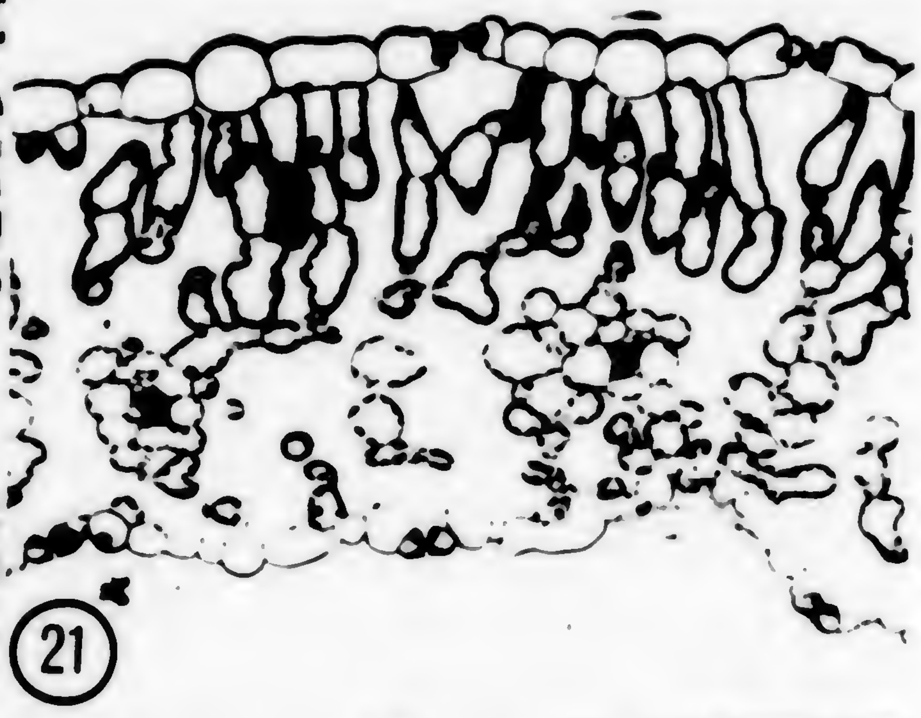
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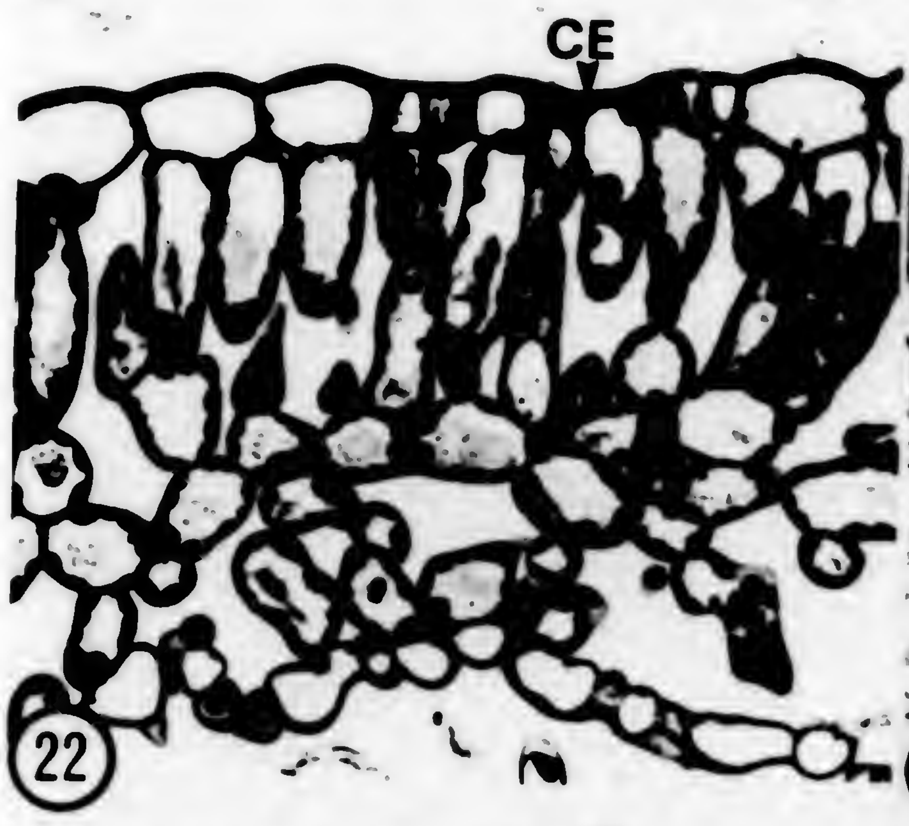

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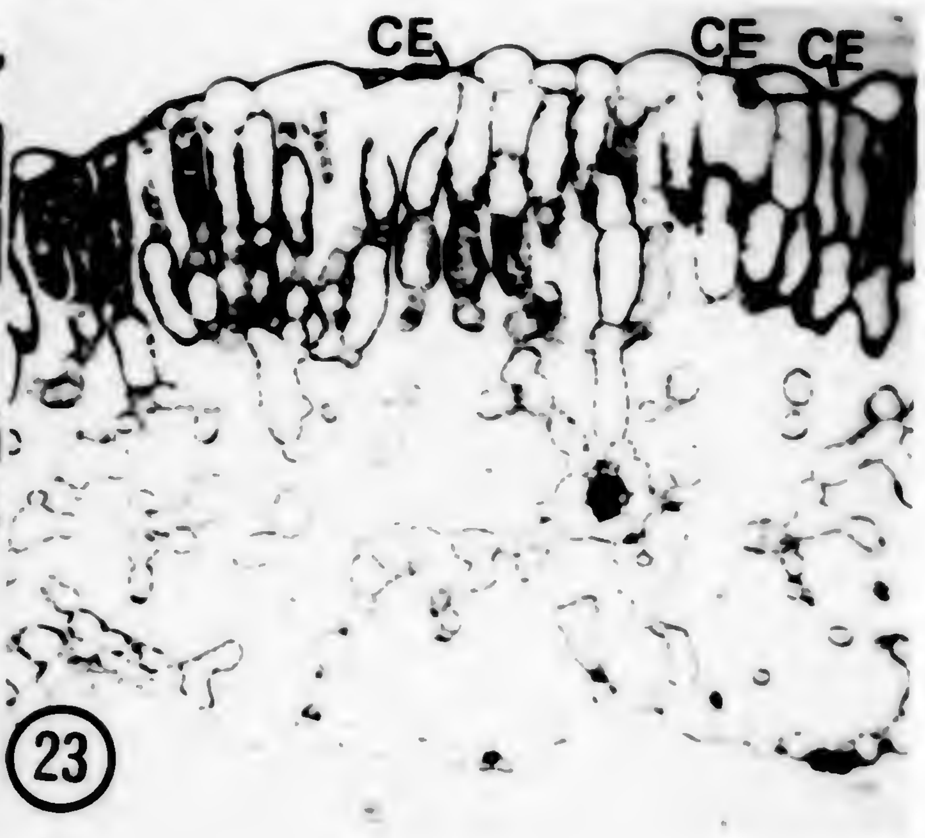

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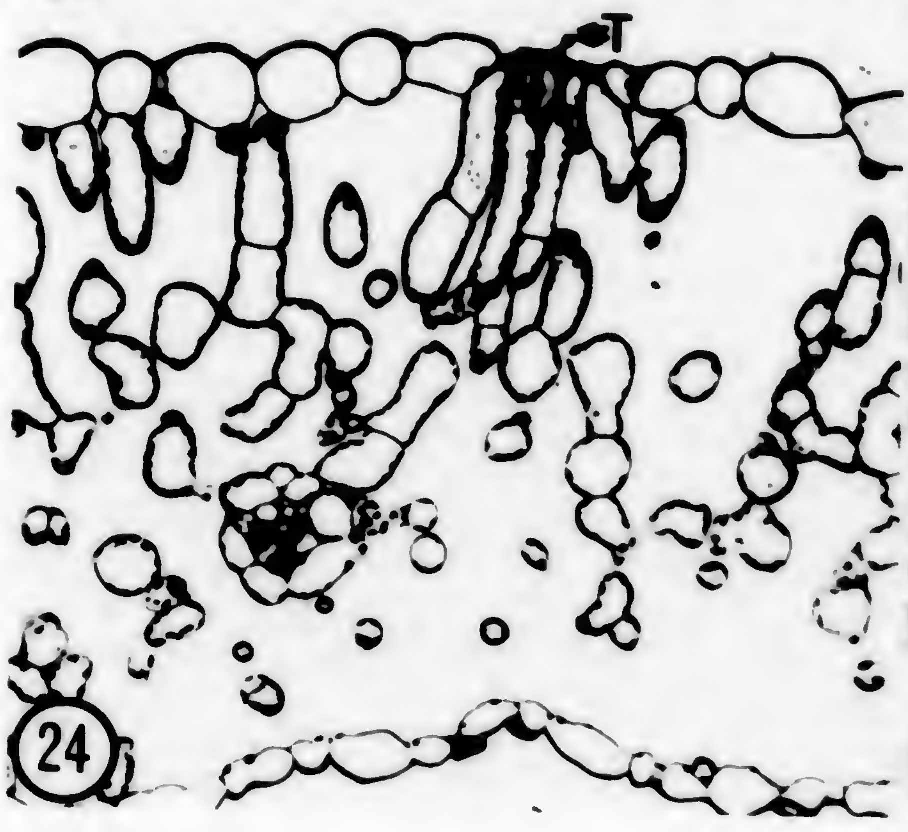

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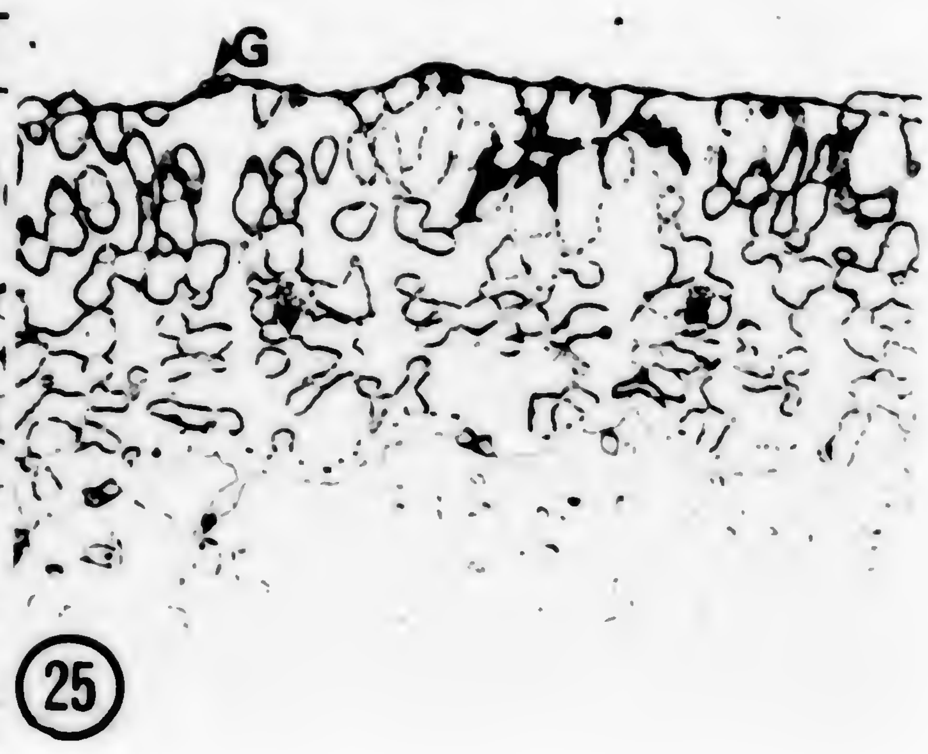

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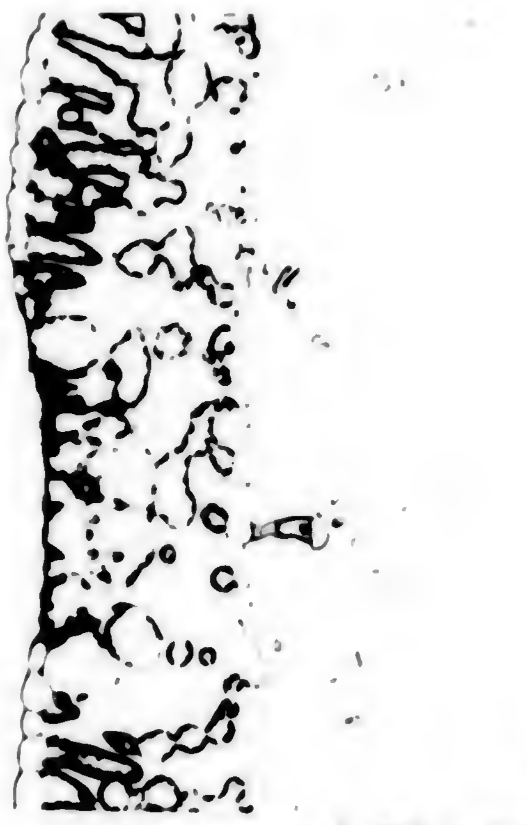
Plates 26 - 30. Cross sections of leaves of Smoking Hills (Plates 26, 27 29 and 30) and Alaskan (Plate 28) *Artemisia tilesii*, characteristic of Stage II in lesion development, in response to simulated acid rain exposure of pH 2.5. Plate 26. Partial collapse of cells of the adaxial epidermis overlying an area in which palisade parenchyma (P) cells exhibit hypertrophy (abnormal cell enlargement is indicated by arrows) in response to simulated acid rain exposure (X 148). Plate 27. Hypertrophy, and hyperplasia (abnormal cell proliferation), in response to simulated acid rain in cells of the palisade parenchyma. Newly formed cell walls are indicated by arrows (X 128). Plate 28. Disintegration of palisade parenchyma (p) cells underlying collapsed epidermal cells (X 143). Plate 29. A region of hyperplastic activity in the palisade mesophyll. Note the decrease in intercellular space in this region and the distortion of the columnar arrangement of the palisade mesophyll (X 121). Plate 30. Collapse of a large area of the adaxial epidermis. Cells of the palisade parenchyma exhibit hypertrophy and hyperplasia. Both of these responses have caused the leaf surface to become elevated (arrows) (X 130).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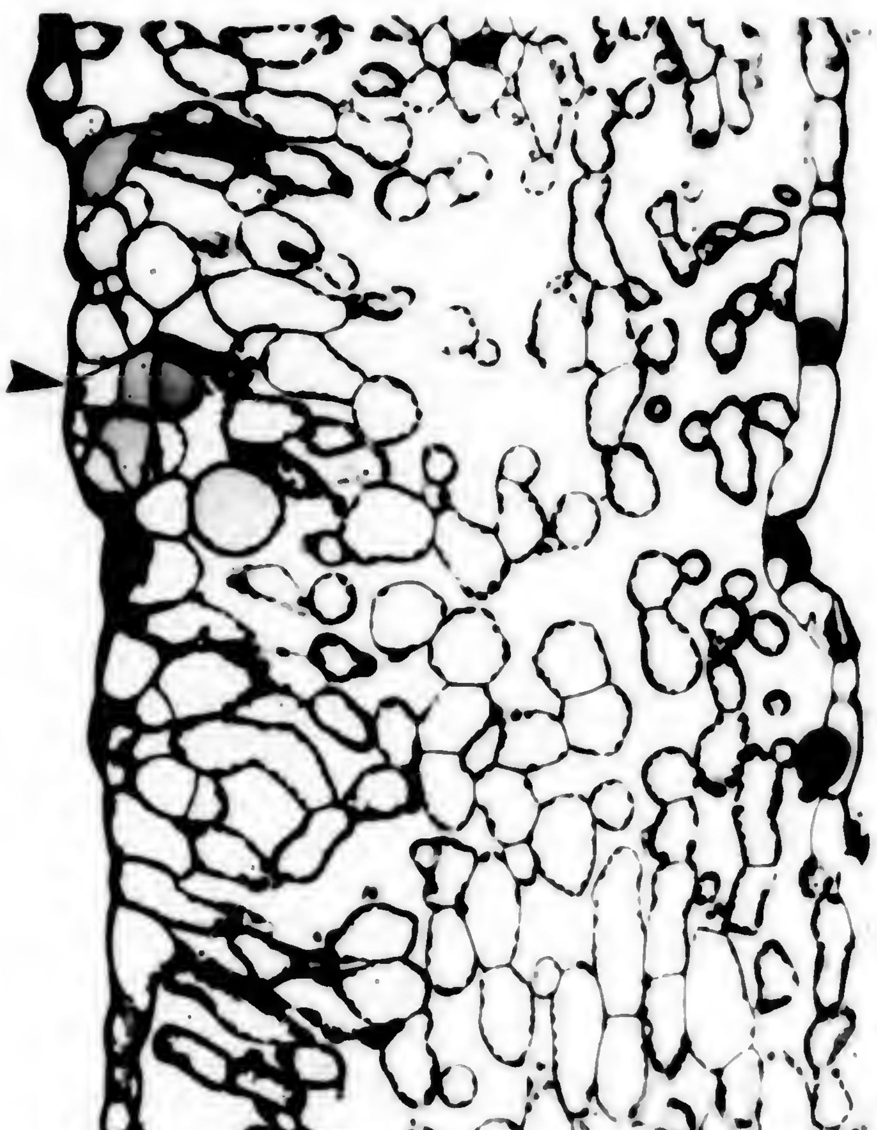
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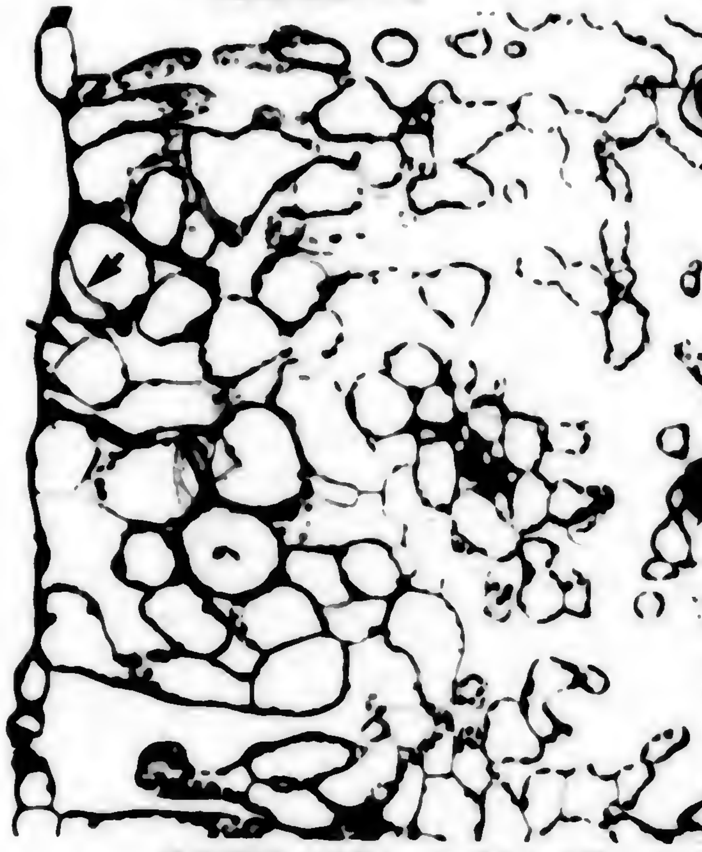
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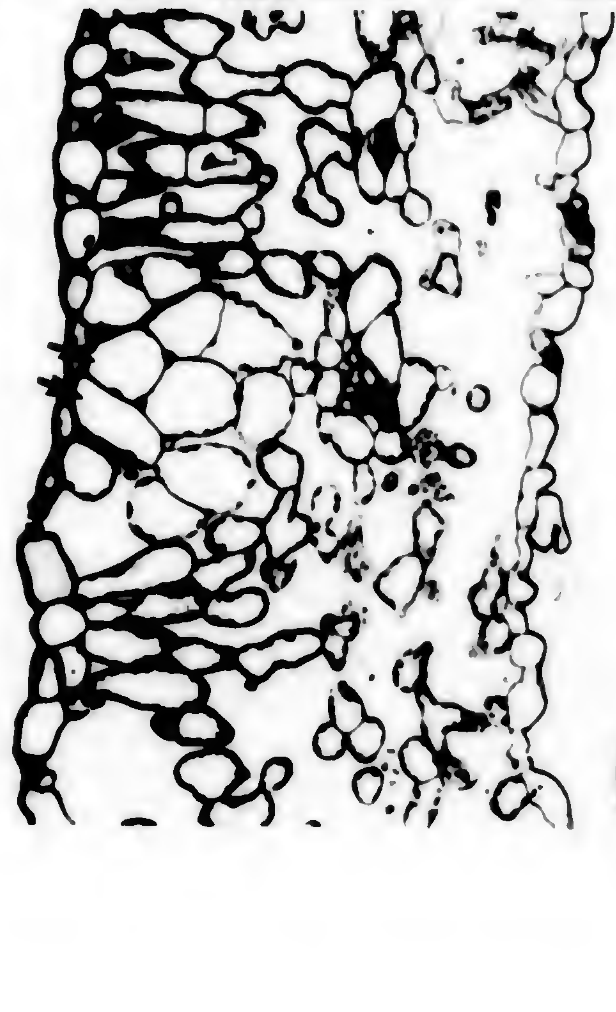
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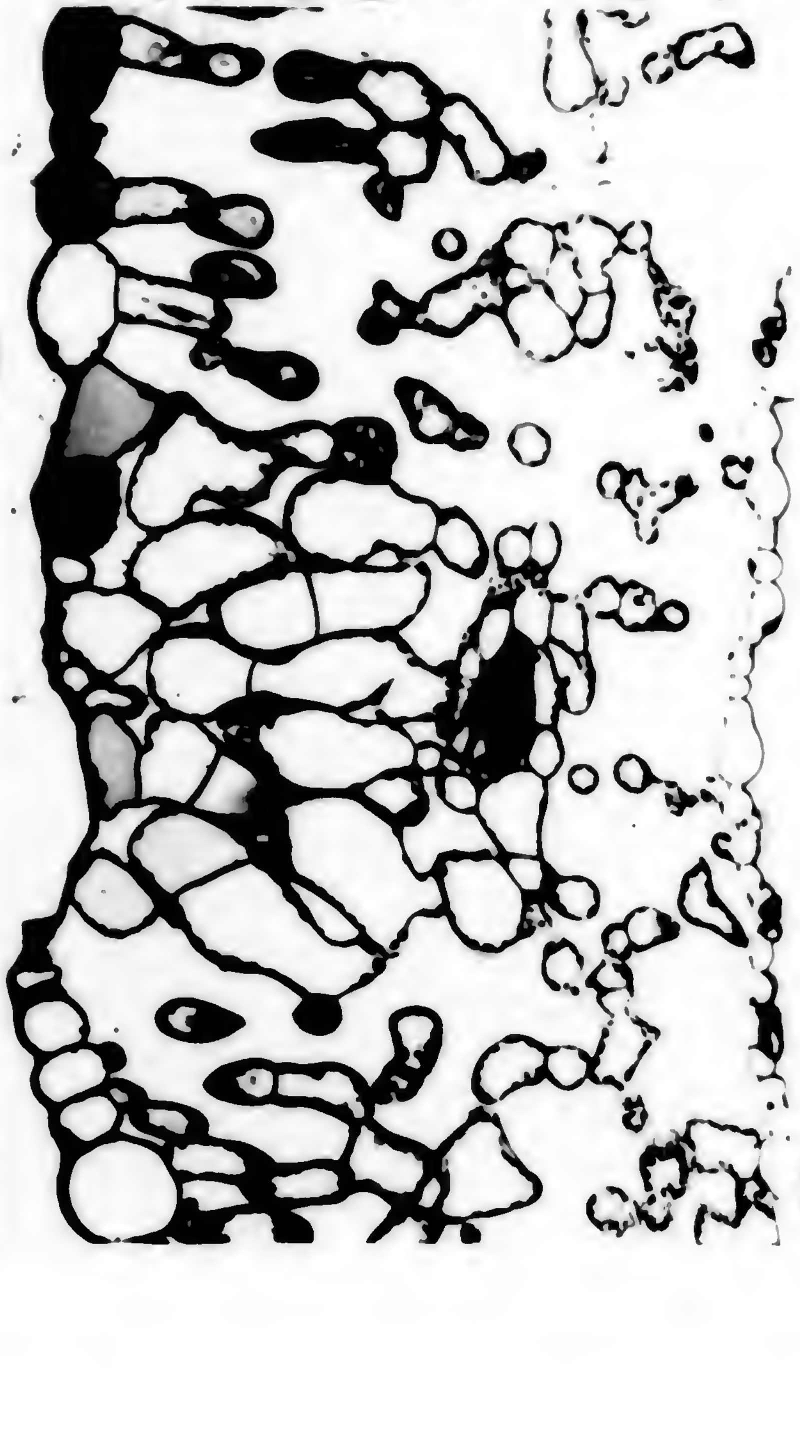
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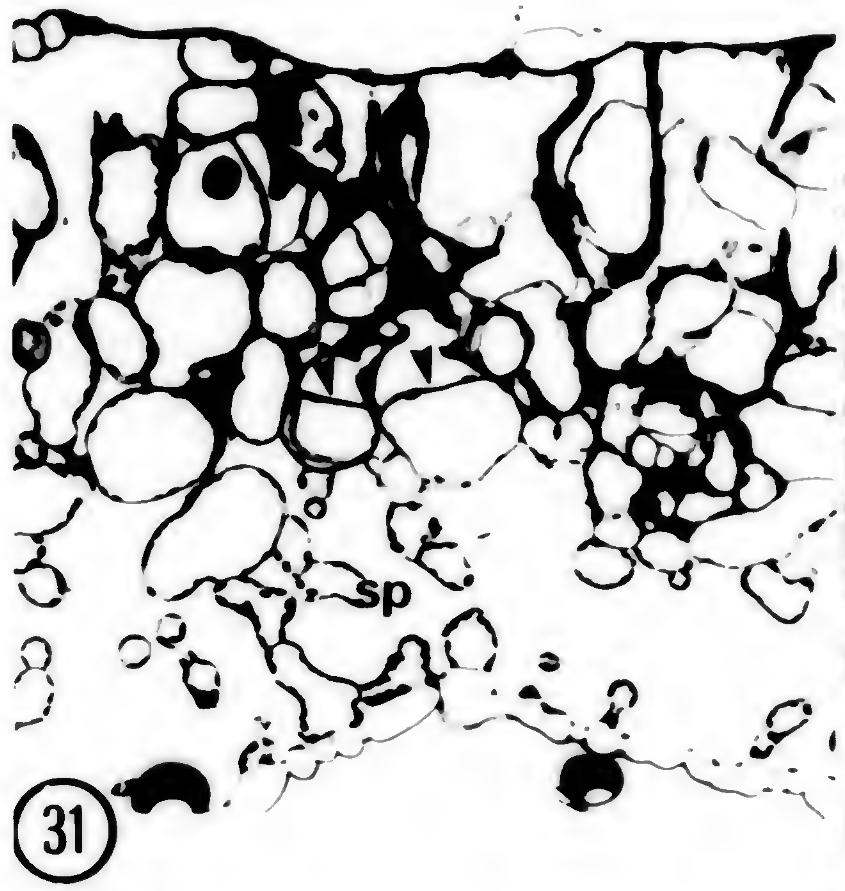
Plates 31 - 35. Cross sections of leaves of Smoking Hills *Artemisia tilesii*, characteristic of Stage III in lesion development, in response to simulated acid rain exposure of pH 2.5. Note collapse of both epidermal and palisade parenchyma cells to form a depression on the leaf surface. Hypertrophy of spongy parenchyma cells (sp) occurs and to a lesser extent hyperplasia, as shown by the presence of new cross-walls (arrows). Magnifications of plates 31 - 35 are (X 136, 111, 129, 136, 130, respectively). Plate 35. Elevation (E) of the leaf surface over an area of extensive cell division and cell enlargement in the mesophyll cells. Note the high staining affinity of the injured cells in comparison with healthy cells, and the reduction of intercellular space caused by hypertrophy and hyperplasia.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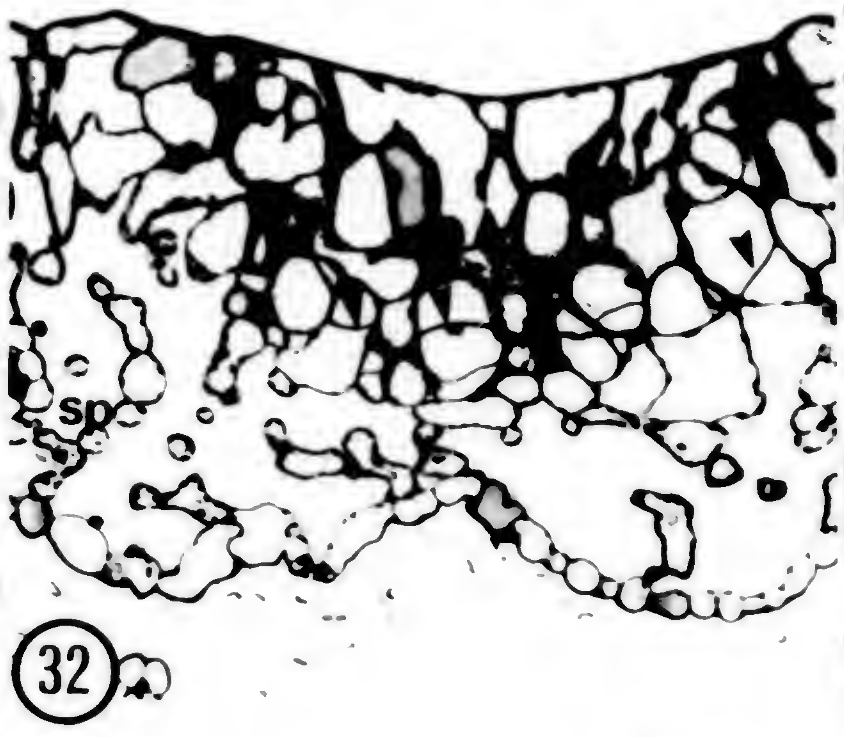
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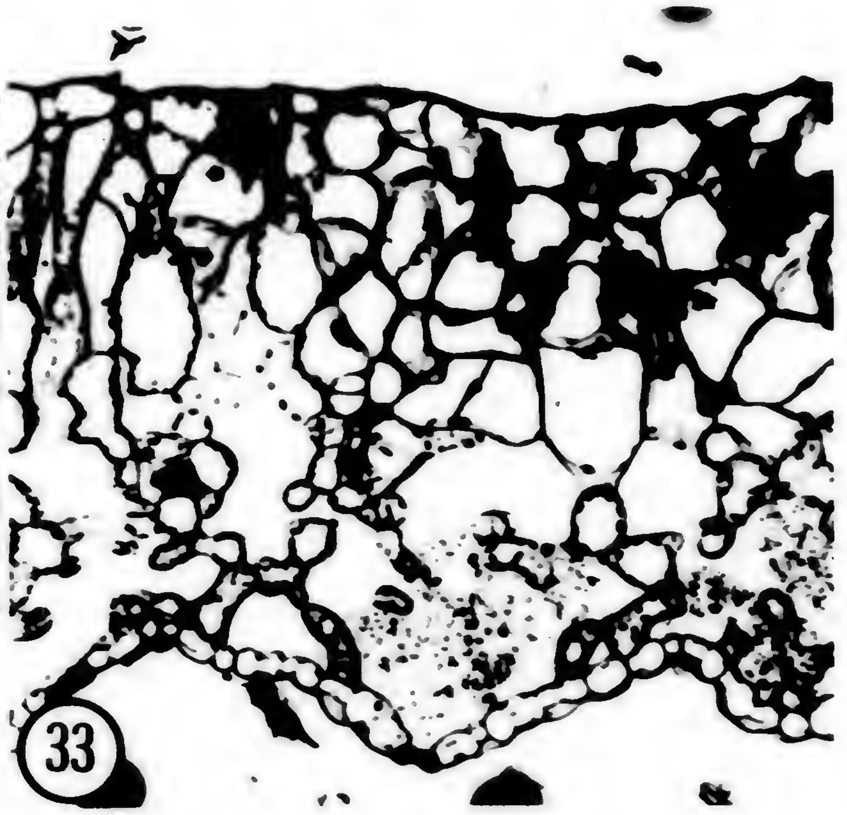
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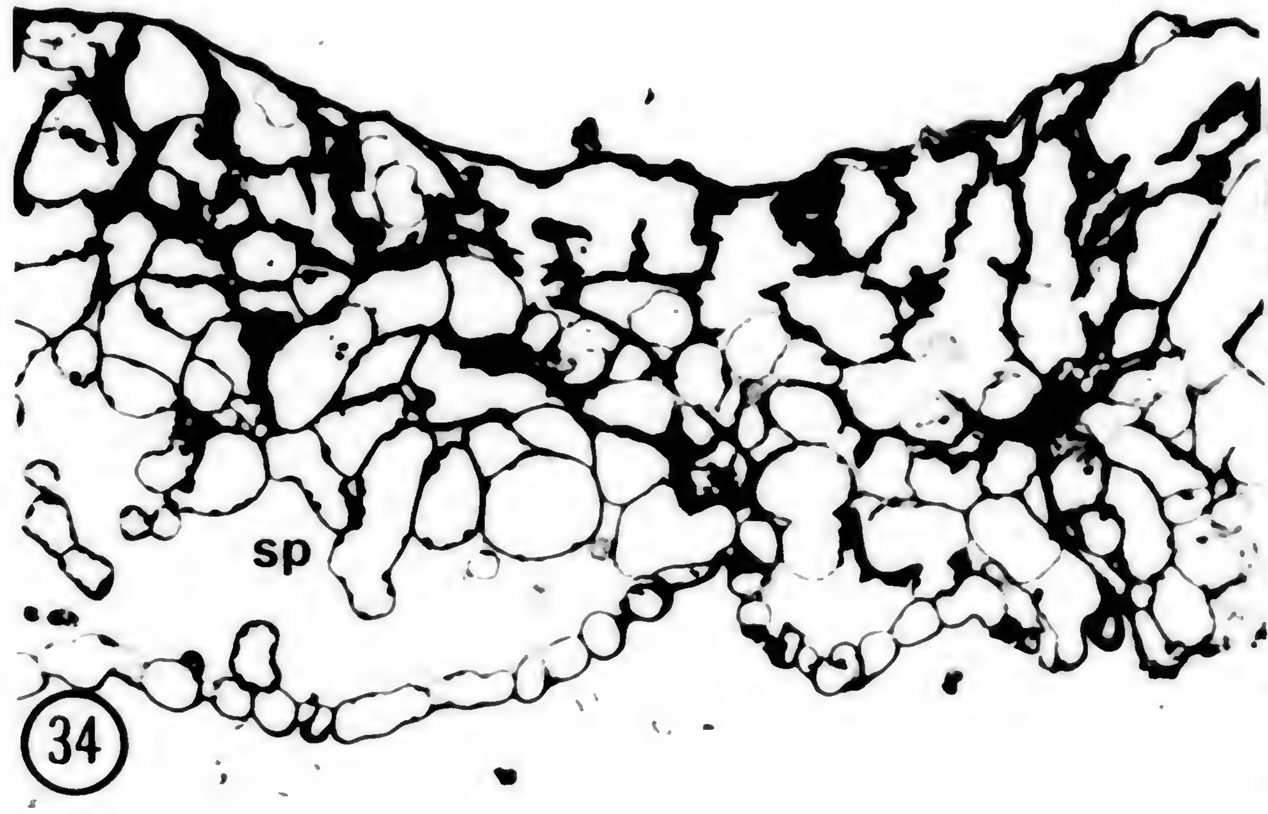
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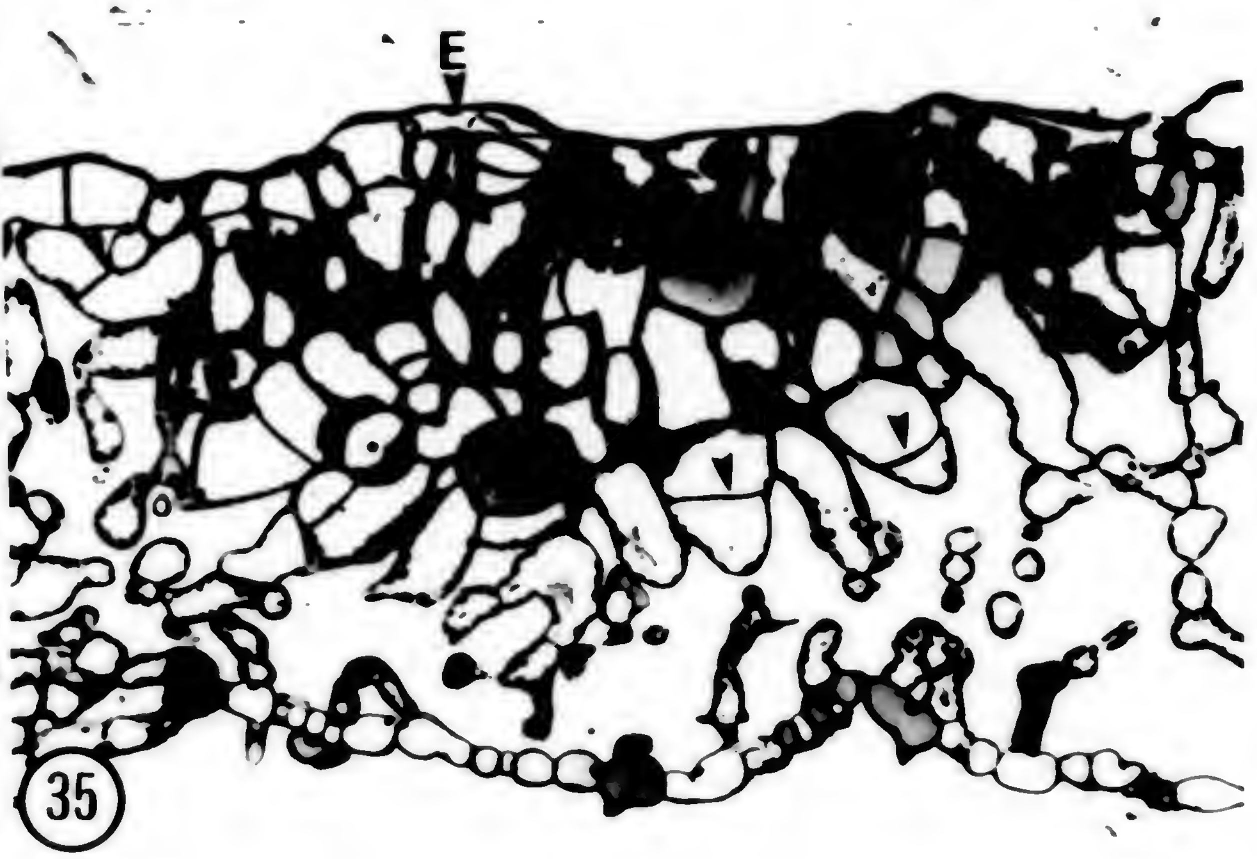
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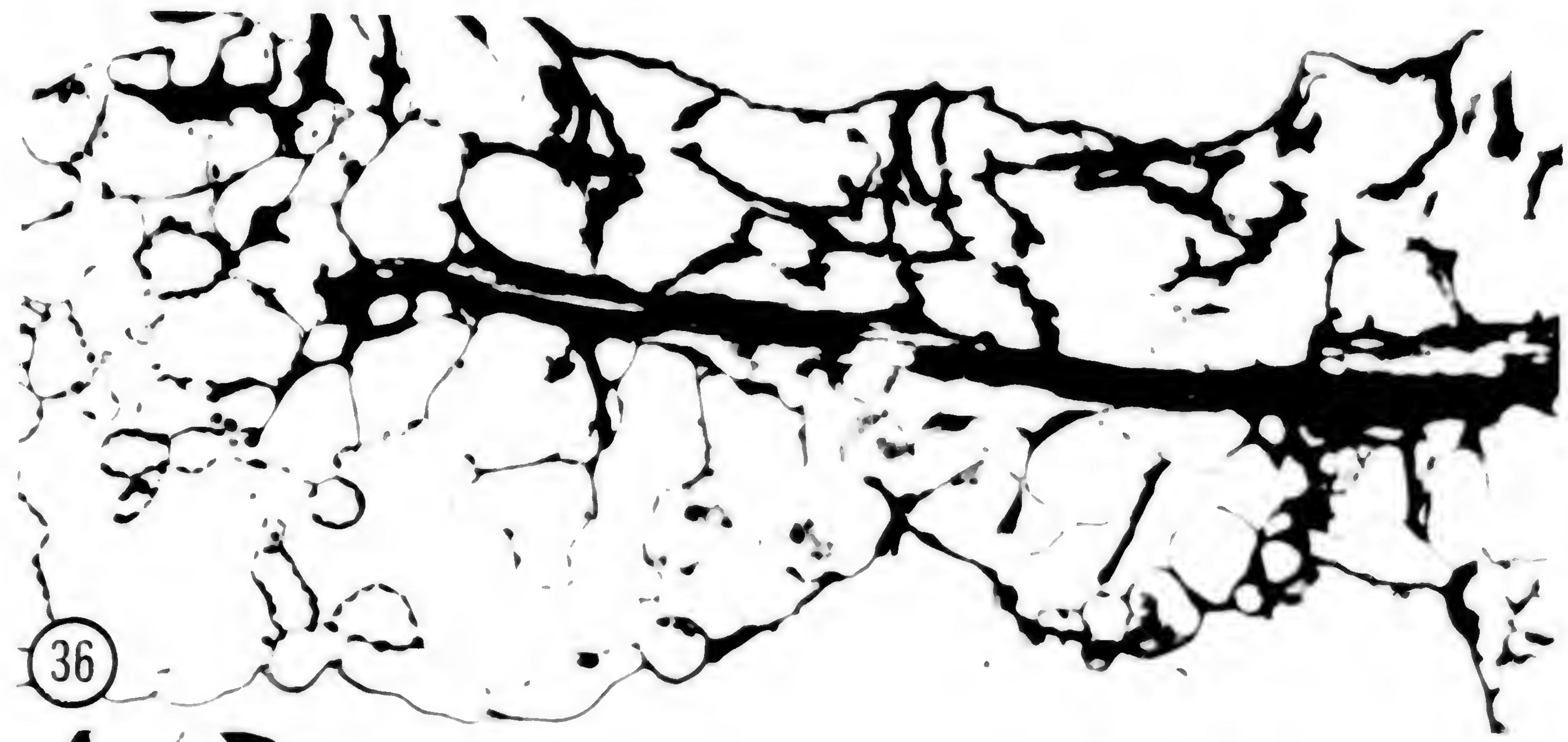
Plates 36 - 38. Leaf cross sections of Smoking Hills *Artemisia tilesii* after several exposures to acidic rainfall of pH 2.5. These leaves are characteristic of Stage IV in lesion development, in which all of the various cell layers have collapsed. Note the damage to vascular tissue (V) shown in Plate 37. Magnifications (X153, X197, X200).



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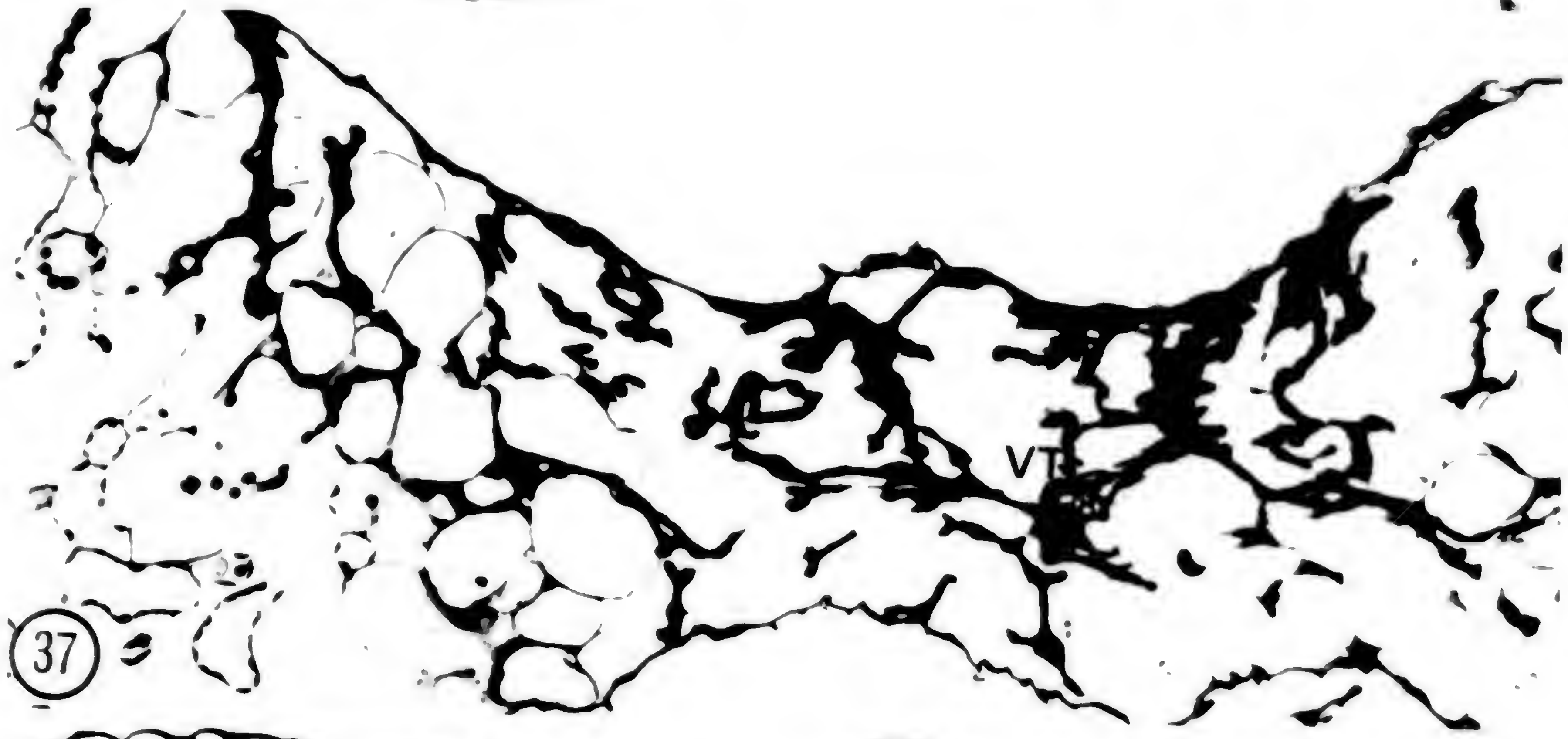

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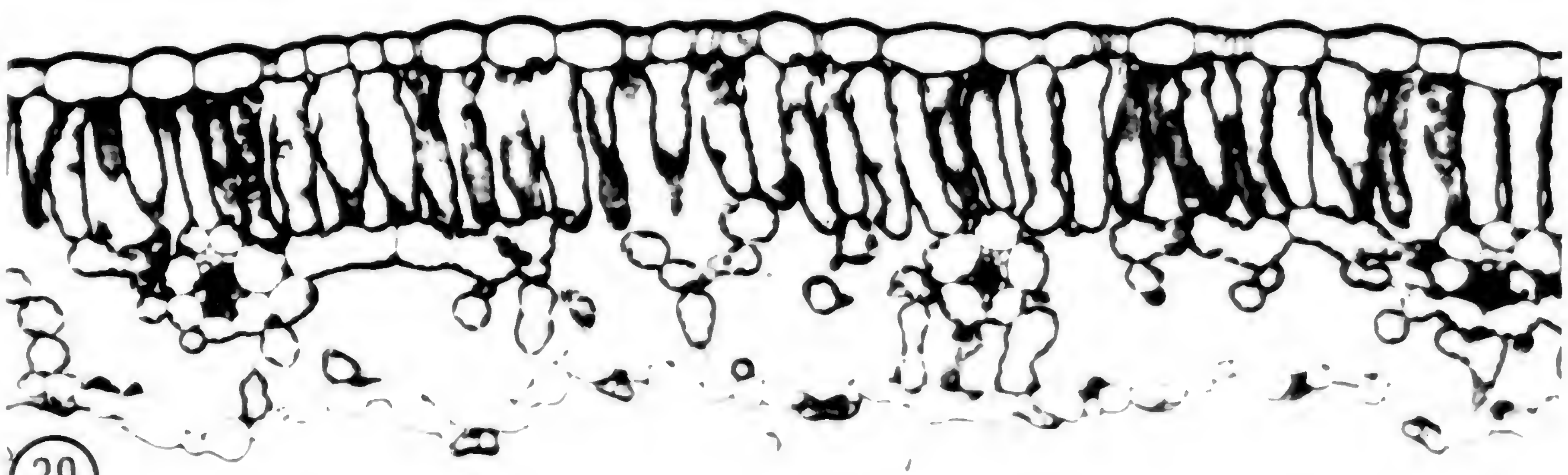
Plates 39 - 41. Leaf cross sections of Artemisia tilesii (Alaskan population) illustrating "Pathway 2" in lesion development. Plate 39. Normal uninjured leaf (X 212). Plate 40. Hypertrophy (indicated by arrows) occurring in cells of the spongy parenchyma in the absence of damage to the adaxial epidermis. Note cells of the adaxial epidermis overlying the injured mesophyll cells are more darkly stained than adjacent epidermal cells (X 205). Plate 41. Hyperplasia, indicated by the presence of nuclei (n) in newly divided cells, occurring in both the palisade and spongy mesophyll. No injury has occurred to overlying cells in the adaxial epidermis. However, these cells are more darkly stained than adjacent epidermal cells. Note the reduced amount of intercellular space in this region (X 204).



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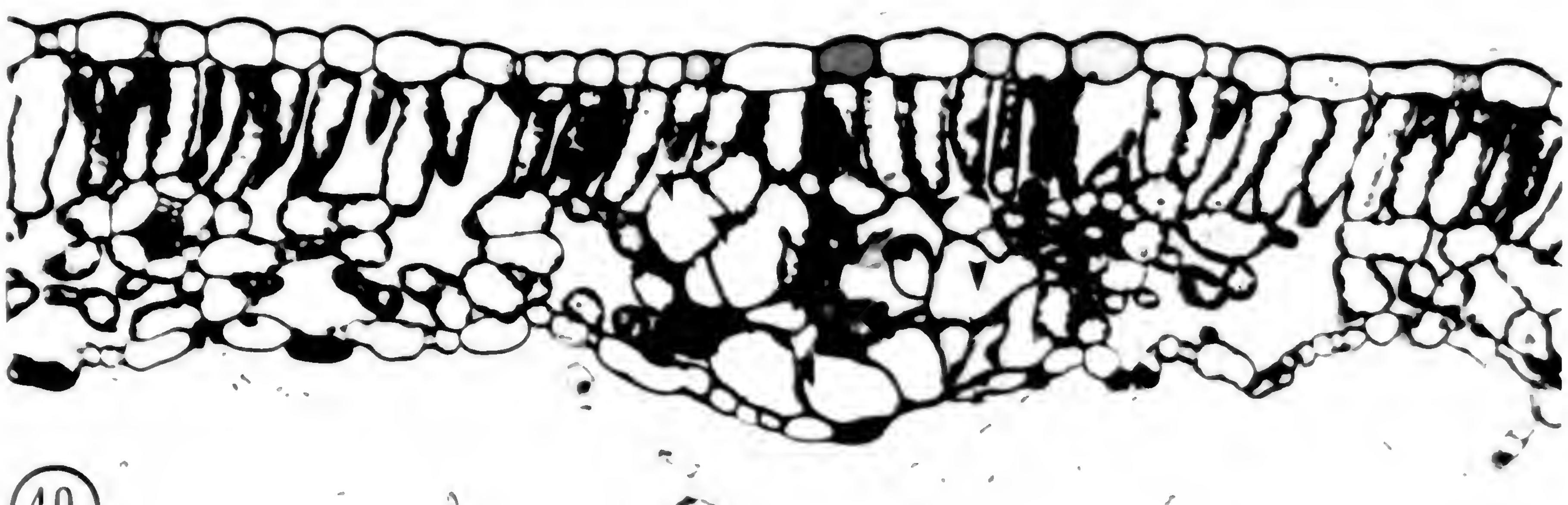

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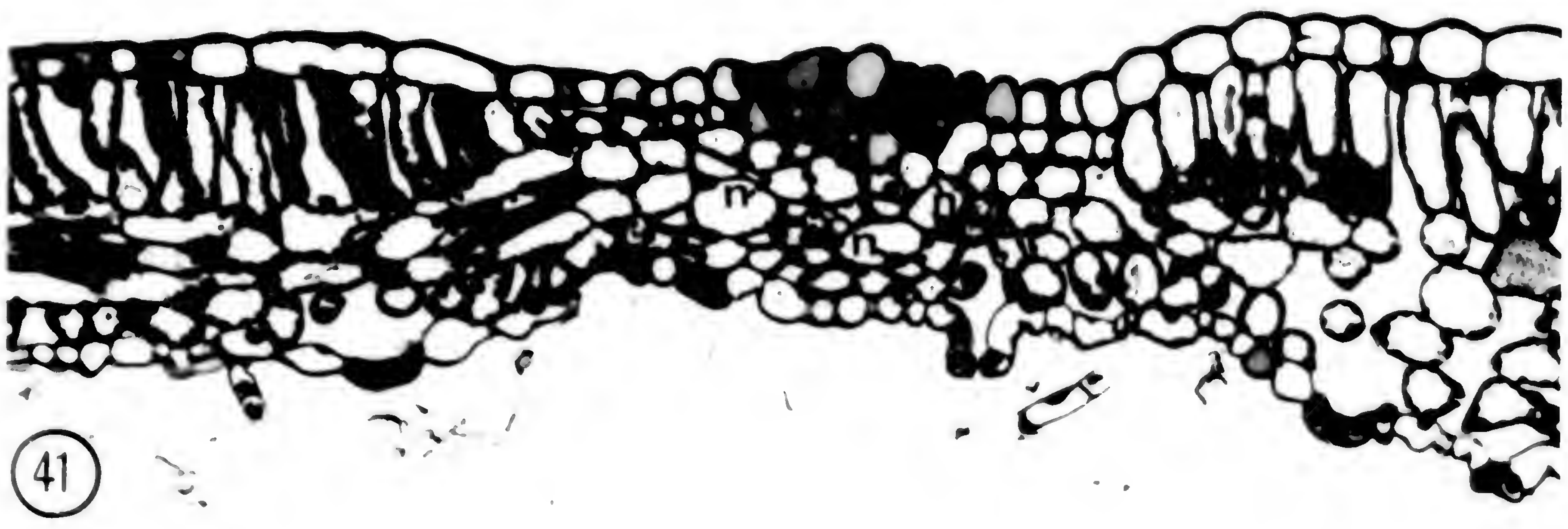

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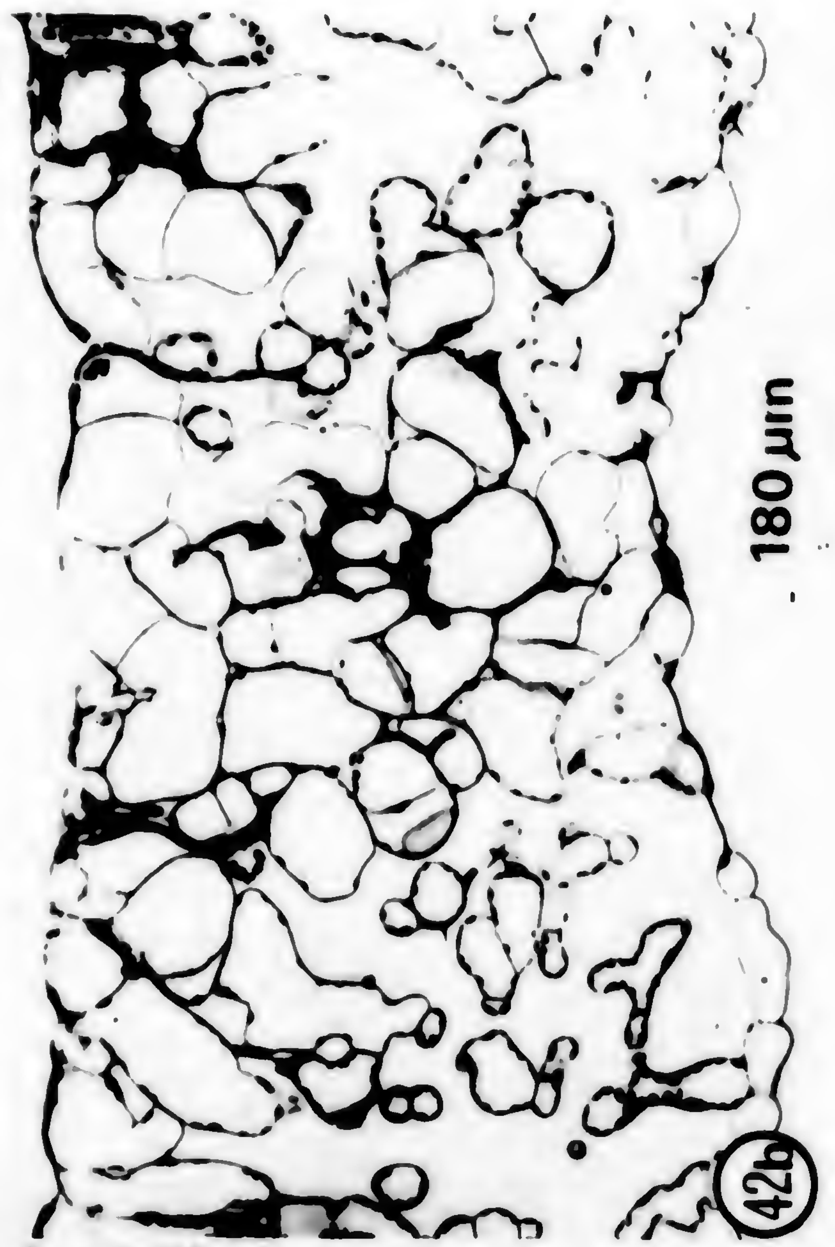
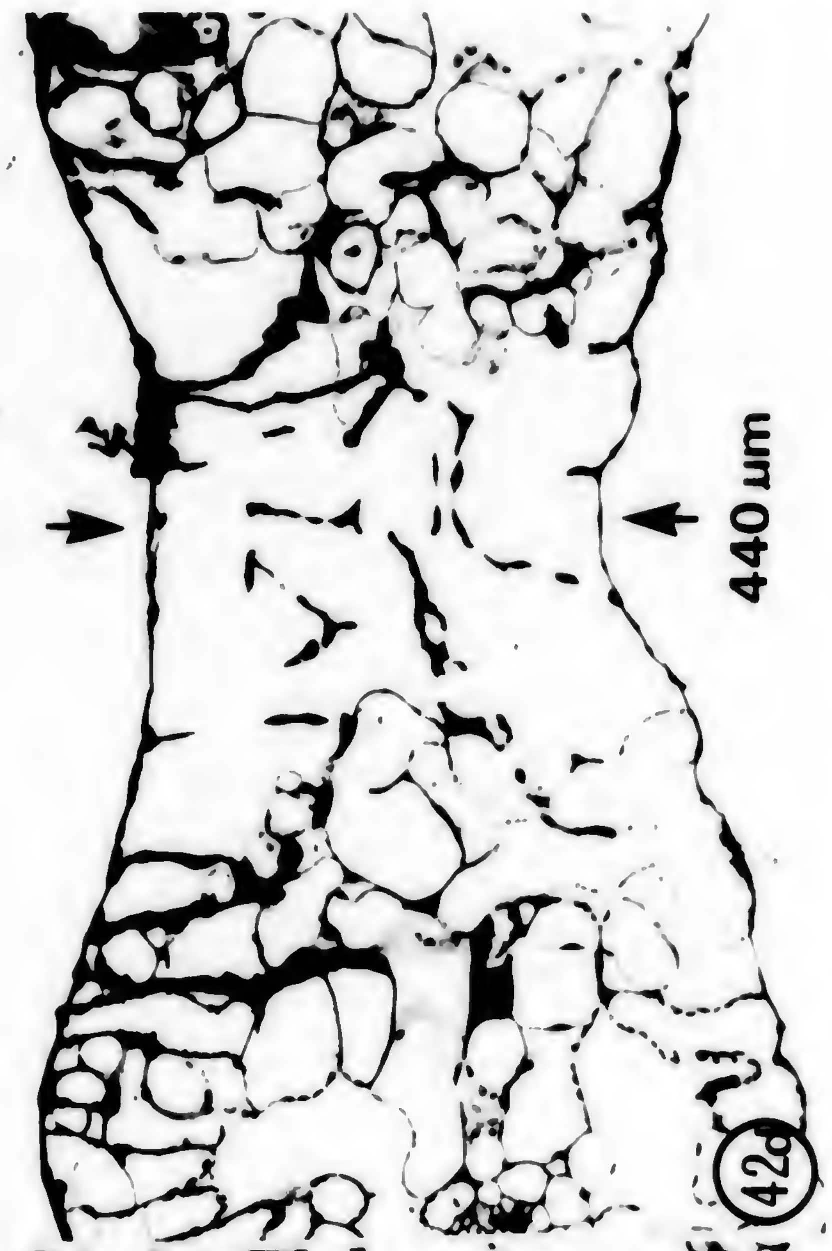
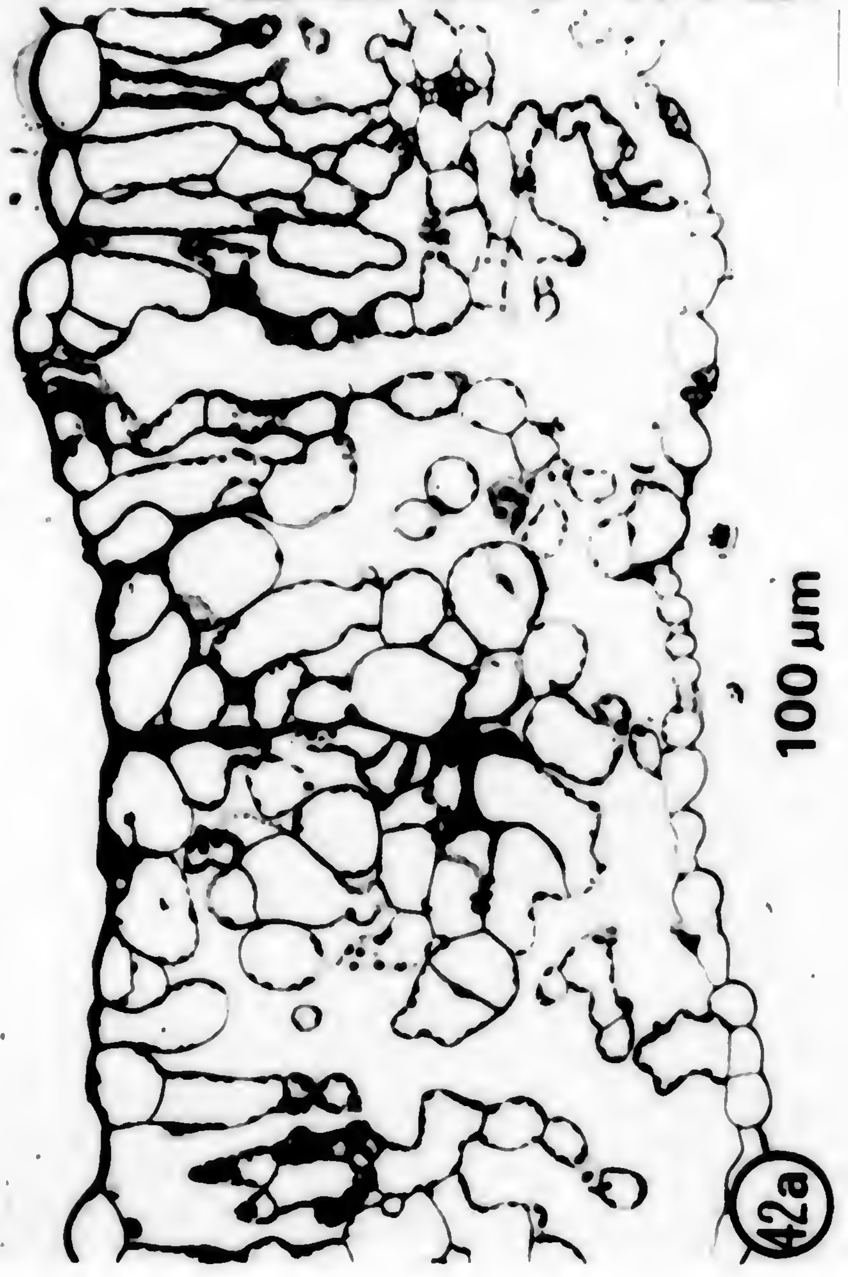
Plates 42a - h. Sequential cross sections taken across a lesion in a Smoking Hills leaf of *Artemisia tilesii* exposed to acidic rain of pH 2.5 illustrating the 3-dimensional nature of lesion development. Changes in the severity of injury that occur across the lesion as seen in these micrographs can be compared with the cell size changes graphed in Figures 14a - e. Distances marked on the micrographs refer to the distance of the section from an uninjured edge of the lesion.

Plate 42a. At 100 µm from the edge of the lesion hypertrophy and hyperplasia are evident in palisade and spongy parenchyma cells beneath an area in which epidermal cells have collapsed (X 137). Plate 42b. 180 µm from the lesion edge. The region of hypertrophy and hyperplasia is more extensive (X 120). Plate 42c. 260 µm from the lesion edge. Collapse of palisade parenchyma cells has occurred to form a depression in the leaf surface. Plate 42d. 440 µm -- the center of the lesion. All of the cell layers have collapsed (indicated by arrows) in an area surrounded by a region of cells exhibiting hypertrophy and hyperplasia on either side, separating the damaged tissue from normal healthy tissue (X 147).


42b
180 µm


42d
440 µm


42a
100 µm

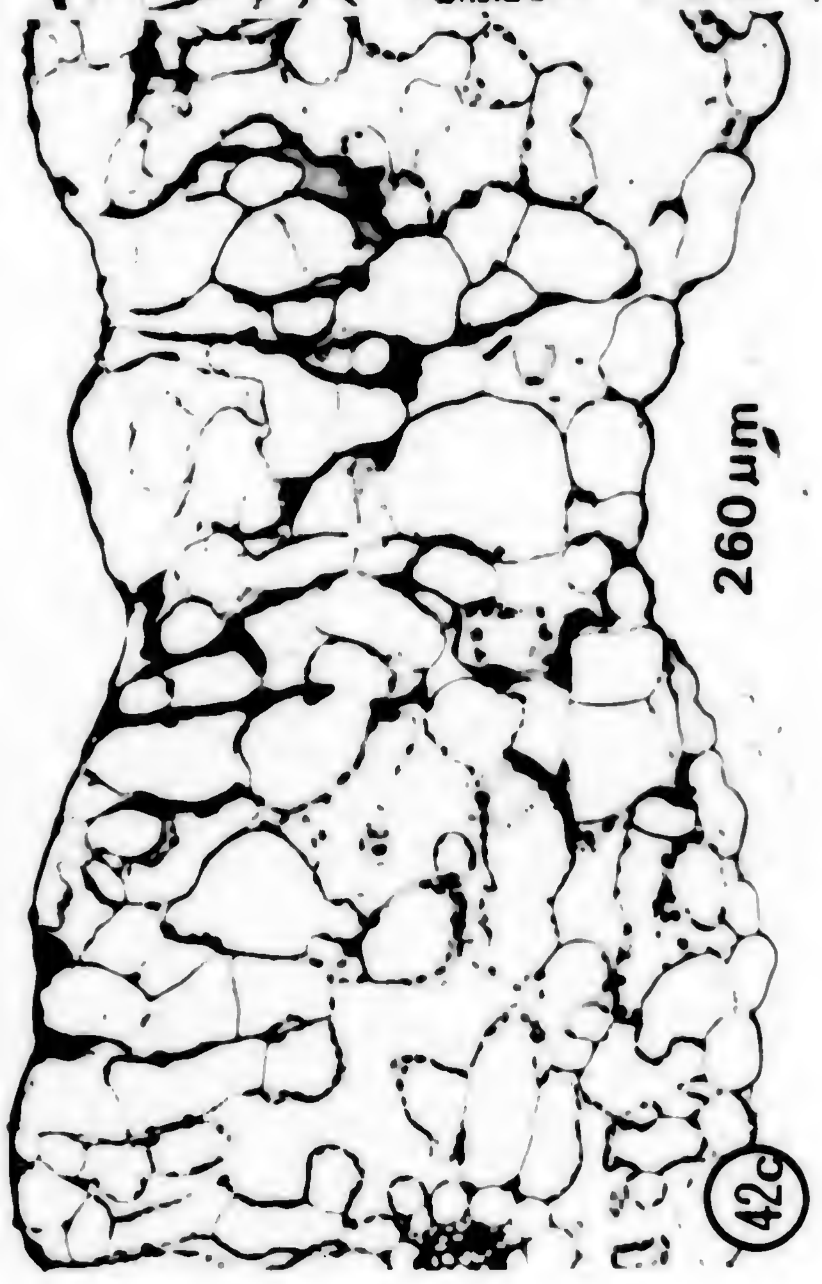
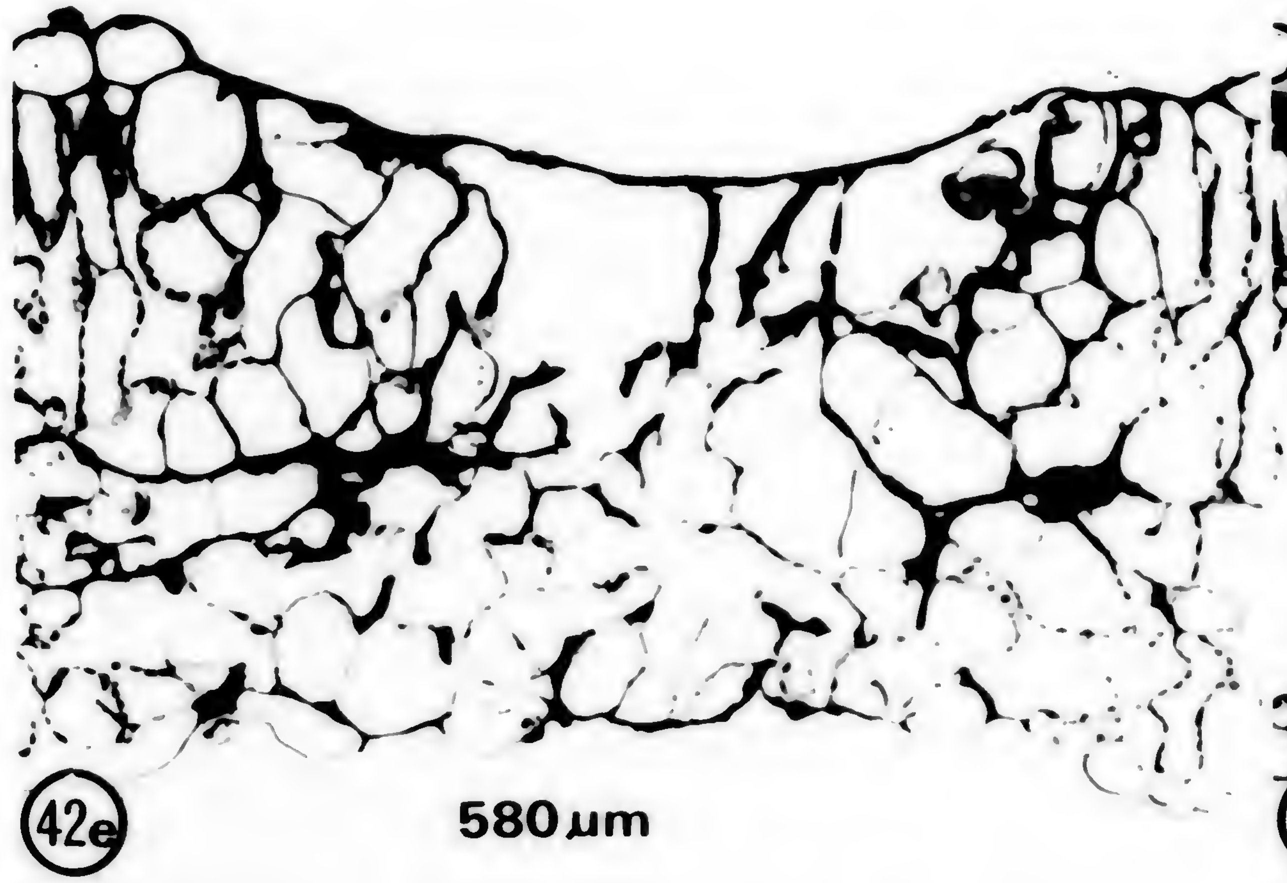
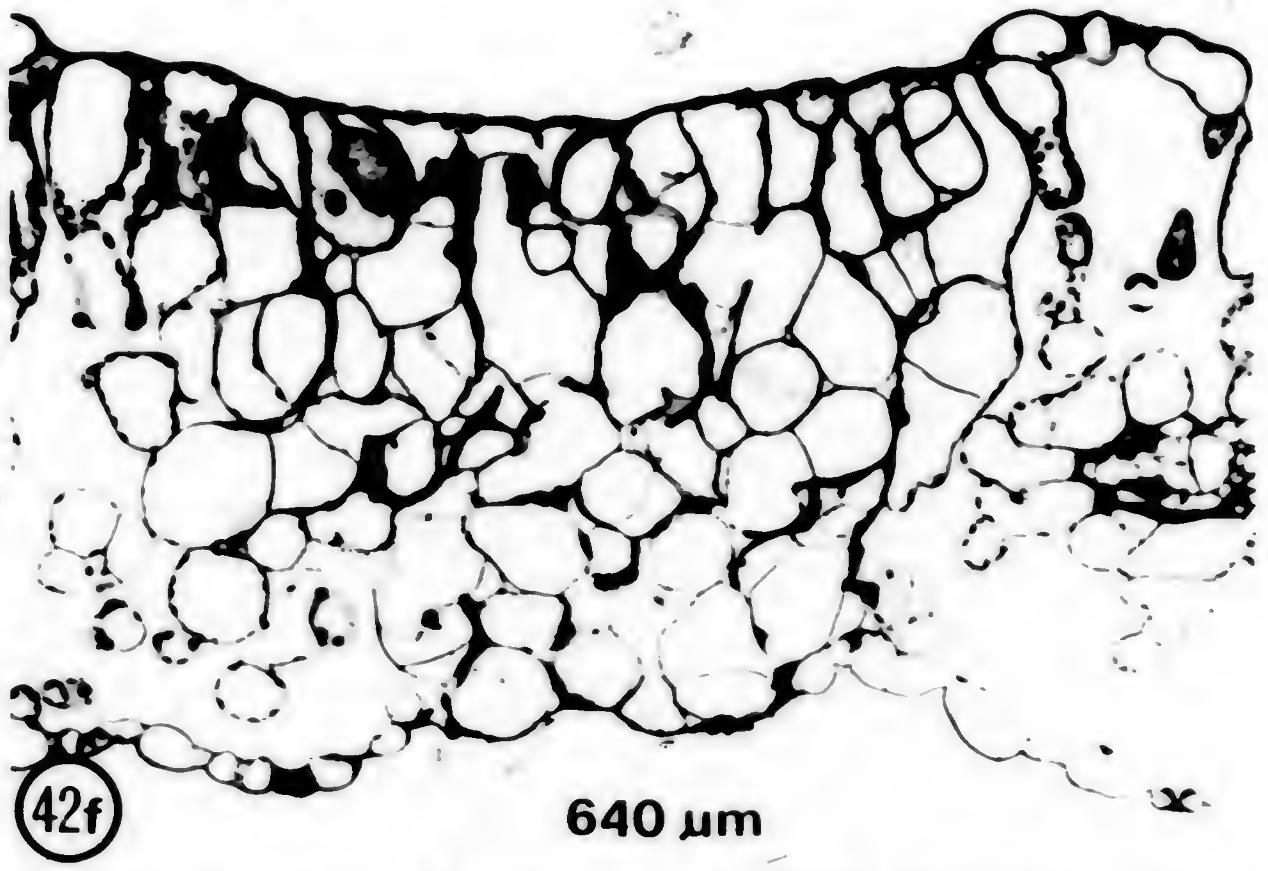
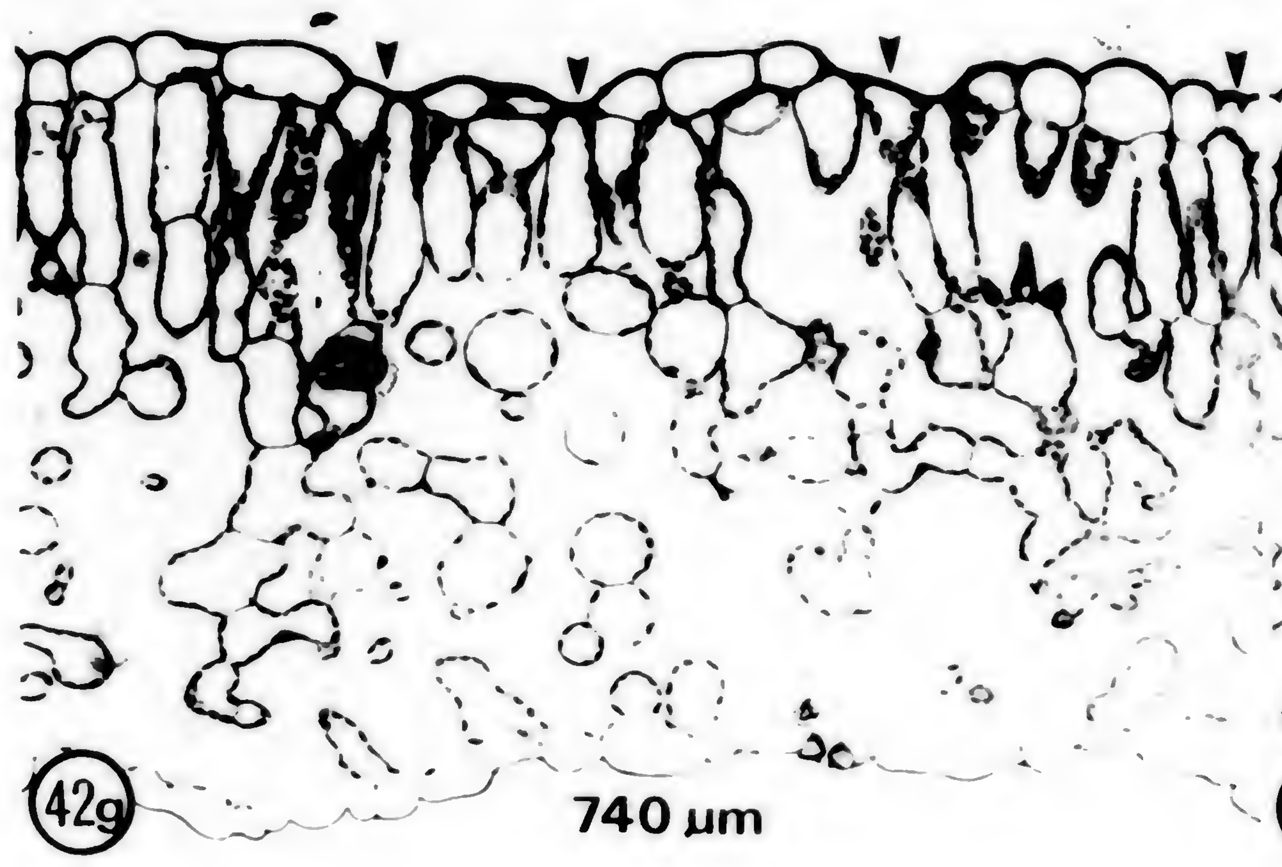
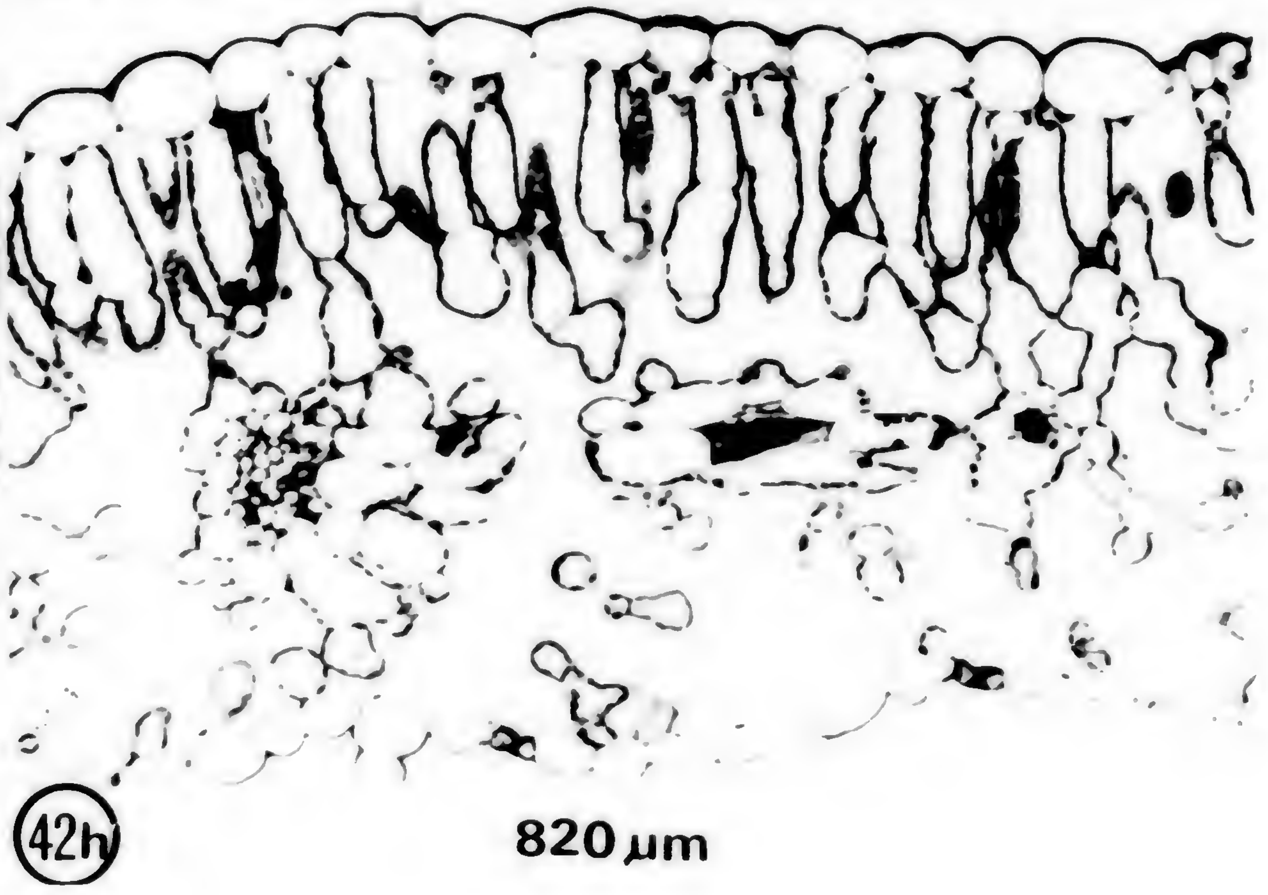

42c
260 µm

Plate 42e. 580 µm from the lesion edge. Collapse of palisade parenchyma and epidermal cells. Hypertrophy and hyperplasia evident in spongy parenchyma in the area of the lesion (X 149). Plate 42f. 640 µm from the lesion edge. Collapse of the adaxial expidermis overlying an area in which hypertrophy and hyperplasia are evident in the mesophyll tissue (X 147). Plate 42g. 740 µm from the lesion edge. Collapse of a small number of epidermal cells (arrows) (X 139) . Plate 42h. 820 µm from the lesion edge. This is the opposite edge of the lesion and no injury from simulated acid rain is evident (X 145).

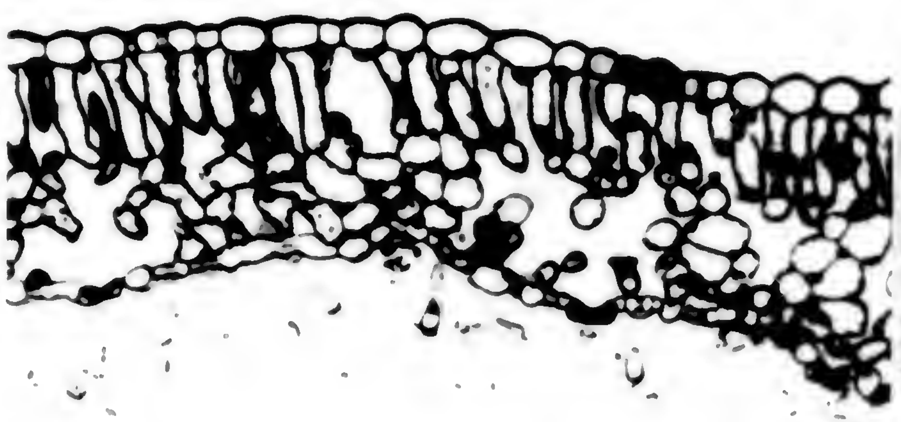

42e
580 µm


42f
640 µm


42g
740 µm

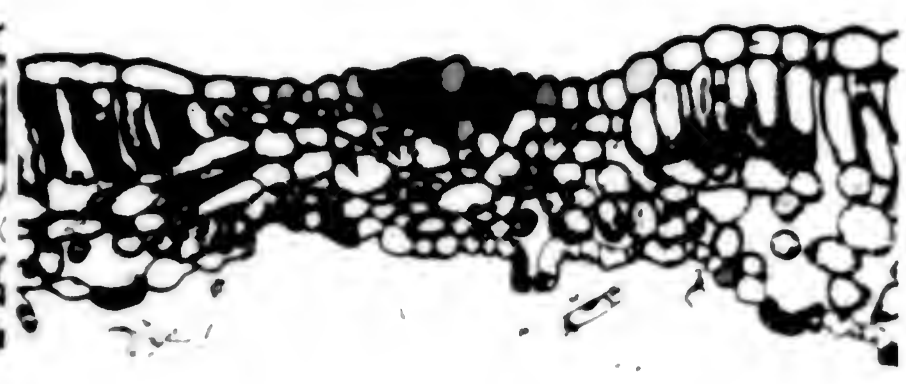

42h
820 µm

Plates 43 a - f. Sequential sections taken across a lesion in an Alaskan leaf of Artemisia tilesii exposed to acidic rain of pH 2.5 illustrating the 3-dimensional nature of lesion development. Changes in the severity of injury that occur across the lesion as seen in these micrographs can be compared with the cell size changes graphed in Figures 15a - e. Distances marked on the micrographs refer to the distance of the section from an uninjured edge of the lesion. Plate 43a. Uninjured leaf tissue (X 125). Plate 43b. 200 µm from the lesion edge. Hyperplasia (indicated by arrows) has occurred in the absence of injury to the adaxial epidermis. Note the reduction of intercellular space in the region of cellular injury (X 130). Plate 43c. 300 µm from the lesion edge. Hyperplasia indicated by nuclei (n) in newly divided cells beneath an area in which cells of the adaxial epidermis have collapsed (X 156). Plate 43d. 440 µm from the lesion edge. Collapse of all cell layers to form a narrow band of tissue. This area is separated from normal healthy cells by an area in which extensive hyperplasia has occurred resulting in a reduction in intercellular space (X104). Plate 43e. 540 µm from the lesion edge (lesion center). Note presence of nuclei (n) in newly divided cells on either side of a band of severely collapsed tissue (X 138). Plate 43f. 900 µm -- the far side of the lesion. None of the cell layers is injured in this micrograph (X 125).



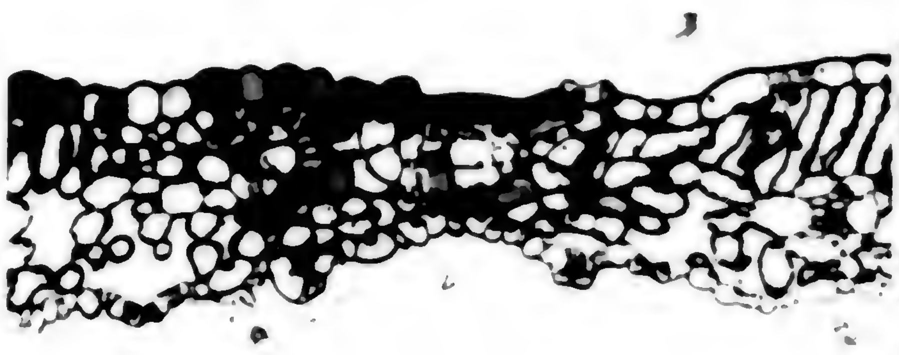
0 µm

43a



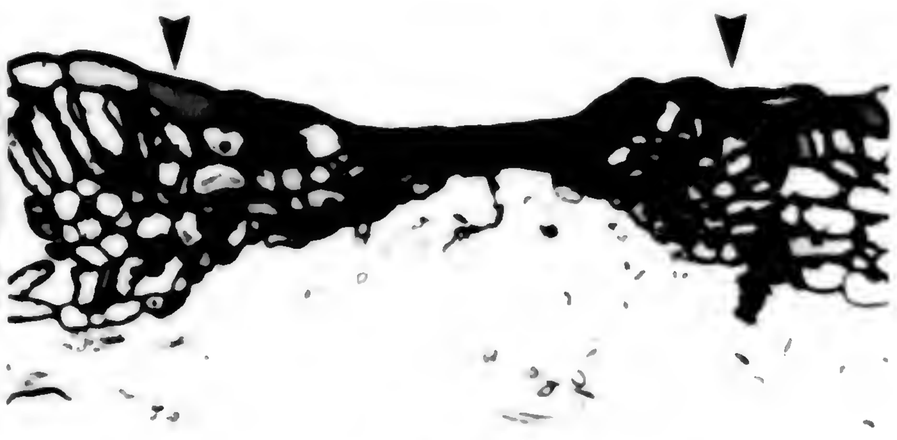
200 µm

43b



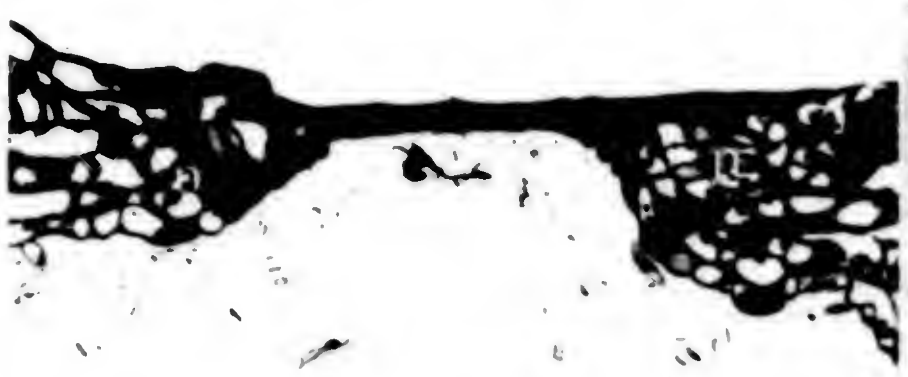
300 µm

43c



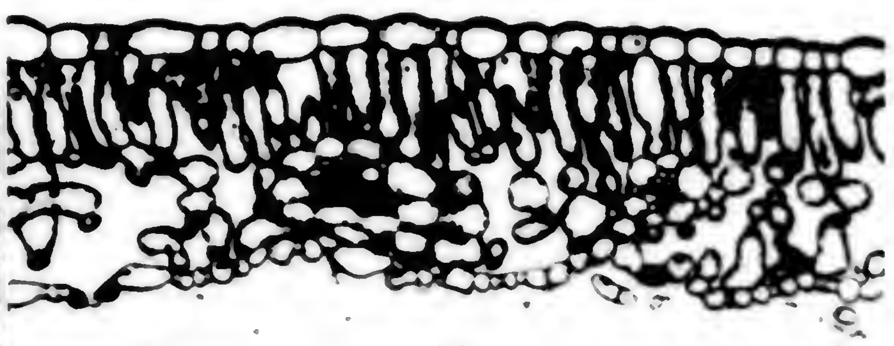
440 µm

43d



540µm

43e



900 µm

43f

2. Histological responses of foliage of Artemisia tilesii to simulated acid rain

a. Comparison of normal leaf anatomy of Artemisia populations

i. Leaf anatomy of Smoking Hills Artemisia

The mature leaf of Smoking Hills plants characteristically has eight to eleven cell layers. These consist of the adaxial epidermis, two layers of columnar, loosely packed palisade mesophyll, four to seven layers of spongy mesophyll and the abaxial epidermis (Plates 20 and 21). Cell dimensions for each of these cell types are given in Table 31. With the exception of the cell width of spongy parenchyma, mean cell height and width of other tissue layers range from 30 - 50% larger than dimensions of corresponding cell types in the leaves of the Alaskan population. The thickness of the lamina ranges from a minimum of 378 µm to a maximum of 419 µm, approximately 2.5 times thicker than leaves of Alaskan plants. Leaf surface features include a complex reticulate minor venation, stomata on both the upper and lower leaf surface (Plates 15 and 21), stalked capitáte trichomes on the upper epidermis (Plate 15) and long multicellular trichomes on the lower epidermis, which produce a dense mat of hair on the underside of leaves (Plate 7). At a magnification of 1000 X both guard cells and subsidiary cells (upper epidermis) are seen to have ridges or folds which extend longitudinally on the guard cells and radially into adjacent subsidiary cells (Plate 16).

ii. Leaf anatomy of the Alaskan population

In contrast to the leaves of Smoking Hills plants, the thin leaves of Alaskan plants, are comprised of from five to seven cell

Table 31. Comparison of the leaf anatomy of Artemisia tilesii from the Smoking Hills and Alaska (n=16).

PARAMETER	SMOKING HILLS $\bar{x}$ ± SE	ALASKA $\bar{x}$ ± SE
Leaf thickness (µm):		
minimum	378.20 ± 15.20	141.35 ± 3.52
maximum	419.36 ± 17.79	162.53 ± 4.50
Cell height (µm):		
upper epidermis	33.56 ± 1.28	16.42 ± 0.57
palisade(1)parenchyma	67.62 ± 2.62	47.53 ± 2.24
palisade(2)parenchyma	53.53 ± 2.81	not present
upper spongy parenchyma	not present	23.34 ± 1.21
lower spongy parenchyma	25.09 ± 1.41	18.22 ± 1.39
lower epidermis	20.83 ± 0.76	11.40 ± 0.43
Cell width (µm):		
upper epidermis	49.15 ± 2.91	23.57 ± 1.30
palisade(1)parenchyma	26.47 ± 1.15	17.88 ± 0.51
palisade(2)parenchyma	29.72 ± 1.28	not present
upper spongy parenchyma	not present	26.59 ± 1.49
lower spongy parenchyma	24.67 ± 0.83	22.16 ± 1.17
lower epidermis	29.77 ± 1.24	17.31 ± 0.83
Number of cell layers:		
average	9.97 ± 0.29	5.56 ± 0.14
range	8 - 11	5 - 7
Surface features:		
stomata	upper and lower epidermis	lower epidermis
stalked capitáte trichomes	upper epidermis	upper epidermis
multicellular trichomes	dense on lower epidermis	dense on lower epidermis

layers and range from 141 - 162 µm in thickness. The single layer of palisade parenchyma located below the upper epidermis is composed of compactly arranged columnar cells with small intercellular spaces (Plate 39). The spongy mesophyll usually consists of three layers of cells, loosely arranged, with large intercellular spaces. Cell dimensions (Table 31), are 30 - 50% smaller than for corresponding cell types of leaves of Smoking Hills plants. The pattern of minor venation is complex reticulate. Stomates are absent from the upper epidermis of leaves (Plate 18). As with Smoking Hills leaves, stalked capitate trichomes are present on the upper epidermis, while the lower epidermis is covered by a dense mat of multicellular trichomes (Plate 19).

Thus, the populations differed in leaf width, number of cellular layers in the leaves, number of layers of palisade mesophyll, the amount of intercellular space, cell dimensions and in the locations of stomates.

b. Description of histological responses to simulated acid rain -- two pathways in the development of lesions

Lesion development was observed in cross sections of leaves of clone 11 from the Smoking Hills and clone 3 from Alaska after repeated exposure to simulated acid rain. Four stages in lesion development were recognized for leaves treated with acidic rainfall of pH 3.0 and 2.5. Numerous injury symptoms, observed in sectioned leaves which had been previously exposed to acidic rainfall (Table 10), were used in the evaluation of lesion development. No cellular injury was observed in 32 sectioned control leaves.

In most of the sections of injured leaves, lesion development began with the collapse of cells in the adaxial epidermis and was followed by progressive cell damage to the tissues beneath this. This order of appearance of injury was observed in all of the sectioned leaves of Smoking Hills Artemisia and will be referred to as "Pathway 1". A description of this pathway of lesion development follows.

"Pathway 1"

The initial stage (I) in lesion development was the collapse, or partial collapse, of a small number of adaxial epidermal cells (Plates 22 - 24) to form a slight depression on the leaf surface. In some lesions, only one or two cells were injured (Plate 22). Cell damage was accompanied by an increase in staining density in the injured cells. Palisade and spongy mesophyll cells underlying these collapsed epidermal cells showed little or no distortion at this stage.

Some micrographs give the impression that epidermal cell collapse begins at the anticlinal walls (Plate 23), producing cells which are flattened out at the two ends almost to a point, giving them an oval shape. In contrast, uninjured cells are square to rectangular in shape with distinct cross-walls between cells. An alternative interpretation is that whole cells collapse and in doing so influence the shape of adjacent healthy cells (Plate 23). Guard cells (Smoking Hills leaves) were less subject to distortion than other epidermal cells, possibly because of their thicker cell walls. In Plate 25, two guard cells are shown to have maintained their integrity while adjacent subsidiary cells and a large number of epidermal cells in the area have collapsed.

In the next stage of lesion development (Stage II) palisade mesophyll cells directly below an area of collapsed epidermal cells exhibited cell hyperplasia (abnormal cell proliferation) and hypertrophy (abnormal cell enlargement). Again, injured cells had a higher affinity for stain than normal mesophyll cells. Chloroplasts exhibited disintegration or were absent in palisade parenchyma cells in Stage II lesions. Proliferation and enlargement of palisade parenchyma cells, in response to acid injury, caused complete distortion of the shape and also the columnar arrangement of the palisade mesophyll, so that it was not possible to distinguish these layers from the spongy mesophyll. This was also accompanied by a decrease in intercellular space in the lesion area in the upper cell layers. Plates 26 - 30 show lesions characteristic of Stage II.

Stage III in lesion development is characterized by distortion of the spongy mesophyll (Plates 31 -34). Chloroplasts disappeared in spongy parenchyma cells by Stage III in lesion development. Hypertrophy of spongy parenchyma cells was the most frequent cellular response, while cell divisions occurred in spongy parenchyma cells in a smaller number of lesions. Both of these responses contributed to a large reduction in the amount of intercellular space (Plate 35). In Plates 30 and 35, the leaf surface is elevated over an area of underlying extensive cell division and cell enlargement in the mesophyll layers. Cells of the adaxial epidermis overlying this area are completely collapsed. This elevation of leaf tissue, resulting from both cell hypertrophy and hyperplasia has been termed 'gall formation' (Evans et al, 1978; 79; Evans and Curry, 1979; Evans, 1980). At Stage III, palisade parenchyma cells begin to deteriorate. Palisade cells in various stages from hypertrophy to complete collapse occur. The number of collapsed adaxial epidermal cells in Stage III lesions may be as great as thirty.

The final stage in lesion development (Stage IV) is the collapse of all cells, including the abaxial epidermis, with the possible exception of vascular tissues (Plates 36 - 38). The highly resistant vascular tissue showed less apparent injury than did the other leaf tissues and appeared distorted and collapsed only in severely damaged leaves (Plate 37).

Severe lesions are often separated from adjacent healthy tissue by an area in which mesophyll cells have enlarged or divided extensively to reduce intercellular space considerably. Thus, there is a gradation in the amount of damage to leaf tissues evident in sections through a lesion, in addition to that which occurs from the leaf surface toward the interior. Stage IV lesions characteristically have a very severely damaged area in the centre of the lesion, bordered by tissue in progressively earlier stages of lesion development towards the periphery of the lesion. This three-dimensional aspect of lesion development will be examined further in a later section.

"Pathway 2"

In a small percentage of lesions occurring on Alaskan leaves (<10%) an exception to this order of appearance of injury was noted, viz., "Pathway 2". In these lesions, injury (hypertrophy or hyperplasia) was observed in internal mesophyll tissues with no apparent injury to the adaxial epidermis occurring at the time (Plates 40 and 41). Epidermal cells overlying the affected mesophyll area were darkly stained. However, the cells were turgid, and normal in shape. This pattern of initial internal injury is of special interest as to mechanism since the question of hydrogen ion penetration is raised.

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c. Measurements of cell sizes and total lesion size in acid injured and control leaves

Cell size measurements of five tissues were undertaken to quantify the qualitative histological observations made from leaf cross sections, which were documented in micrographs of some of the sectioned material (Plates 20 - 41). The measurements were standardized by first scanning sections covering the entire lesion (for acid damaged leaves) and subsequently measuring cells in the most severely damaged area of the lesion. Leaf tissue was sectioned from plants receiving control rainfall (pH 5.6) and acid rainfall (pH 3.0 and 2.5) after exposure to one, two, four and eight rainfalls.

Analyses of variance of cell height and width of five leaf tissues, for clone 11 from the Smoking Hills and clone 3 from Alaska, showed a significant effect of rainfall pH, but no significant effect due to the number of rainfalls applied in all but 5 of the 20 tests, and no significant interaction ($p<0.05$; Tables 32a,b; 33a,b). For these five significant cases, Duncans's Multiple Range test showed that leaves given repeated applications of acidic rain yielded cells both smaller than leaves given only one application (e.g. those exposed to 4 sprayings) and larger than leaves given only one application (e.g. those exposed to 8 sprayings)(Table 34). Since the variation in injury was not consistent with the number of exposures, it was therefore deemed appropriate to disregard the cell size data for individual spray applications (Appendix 6) and look at the combined data for samples from all of the rain applications for each pH treatment (Figures 10 and 11).

Using the combined data, analyses of variance of cell height and width of five cell layers revealed a significant effect of the rainfall pH; primarily in the upper cell layers (adaxial epidermis and palisade mesophyll), and in the abaxial epidermis, for both Smoking Hills clone 11 and Alaska clone 3 Artemisia ($p<0.05$, Table 35). Duncan's Multiple Range Test was used to identify those differences in mean cell sizes of the acidic rainfall treatments which were significantly different from the control rainfall. These are marked with asterisks above the histograms (Figs. 10 and 11) showing that, with one exception, the pH 3.0 treatment had no apparent effect on mean cell size, i.e. cell collapse is characteristic of only pH values below 3.0. It is worth noting however, that although these differences are not statistically significant, there is nevertheless a reduction in cell height and width at pH 3.0 compared with the control for virtually every cell layer.

Figures 10 and 11 show only a slight and non-significant cell enlargement in the mesophyll tissues (e.g. height of upper spongy mesophyll, Alaska, clone 3, pH 3.0; height and width of lower spongy mesophyll, Smoking Hills, clone 11, pH 3.0), despite histological observations of mesophyll cells swelling to enormous sizes at an early stage in lesion development. This suggested that a more appropriate way of ordering the data might be by the severity of tissue damage, since within each pH treatment mean cell sizes combined measurements from leaves with no damage and those in various stages of response including enlargement and collapse.

In examining the data when ordered by lesion severity (Figs. 12 and 13), the following cellular responses are apparent:

1) Adaxial and abaxial epidermis -- progressive reduction in cell height and width leading to complete collapse of adaxial epidermal cells in severe lesions.

2) Palisade mesophyll (first layer) -- progressive reduction in cell height. Cell width is unaffected in minor lesions, increases up to 2 fold in moderate lesions and is drastically reduced due to cell collapse in severe lesions.

3) Palisade mesophyll (second layer, Smoking Hills leaves)/upper spongy mesophyll (Alaskan leaves) -- a similar pattern of response for both cell height and width, which are unaffected in minor lesions, increase in size by approximately 25% in moderate lesions and then decrease to almost zero in severe lesions due to cell collapse.

4) Lower spongy mesophyll -- no significant change occurred to either cell height or width, except in severe lesions where cell size is reduced by up to 50%.

Data for the mean thickness and width of lesions (Table 36) shows a relationship between lesions which are more severe (i.e. affecting more cell layers) and lesion size. Lesions in which all cell layers are injured (severe) are approximately four times wider than minor lesions for the Smoking Hills clone and fourteen times wider than minor lesions for the Alaskan clone.

Table 32a. Analyses of variance of the effect of rain pH, and of repeated exposures, on cell height of various leaf tissues of Smoking Hills Artemisia.

* = P<0.05 ** = P<0.01 *** = P<0.001

Source	F Values for cell height				
	Upper epidermis	Palisade mesophyll	Palisade (2)	Lower spongy mesophyll	Lower epidermis
Rain pH	33.93***	30.25***	11.24***	0.75	1.77
No. of exposures	0.89	1.30	4.59**	2.79	1.71
pH*no. of exposures	0.96	0.68	2.71*	2.20	1.41
Replicate leaves	0.86	1.44	2.26	1.84	0.63

Table 32b. Analyses of variance of the effect of rain pH, and of repeated exposures, on cell width of various leaf tissues of Smoking Hills Artemisia.

* = P<0.05 ** = P<0.01 *** = P<0.001

Source	F Values for cell width				
	Upper epidermis	Palisade mesophyll	Palisade (2)	Lower spongy mesophyll	Lower epidermis
Rain pH	29.65***	2.51	4.29*	1.41	6.17**
No. of exposures	2.35	0.87	2.17	0.44	1.03
pH*no. of exposures	0.87	1.15	2.77	1.68	1.77
Replicate leaves	1.14	0.45	1.11	0.29	0.72

Table 33a. Analyses of variance of the effect of rain pH, and of repeated exposures, on cell height of various leaf tissues of Alaskan Artemisia.

* = P<0.05 ** = P<0.01 *** = P<0.001

Source	F Values for cell height				
	Upper epidermis	Palisade mesophyll	Upper spongy mesophyll	Lower spongy mesophyll	Lower epidermis
Rain pH	14.60***	10.18***	2.06	1.31	4.28*
No. of exposures	7.52***	3.62*	0.14	5.06**	1.03
pH*no. of exposures	1.91	1.92	0.42	0.41	1.50
Replicate leaves	1.21	0.35	0.12	0.44	0.39

Table 33b. Analyses of variance of the effect of rain pH, and of repeated exposures, on cell width of various leaf tissues of Alaskan Artemisia.

* = P<0.05 ** = P<0.01 *** = P<0.001

Source	F Values for cell width				
	Upper epidermis	Palisade mesophyll	Upper spongy mesophyll	Lower spongy mesophyll	Lower epidermis
Rain pH	9.17***	6.58**	2.25	0.74	4.45*
No. of exposures	5.25**	1.02	0.18	0.65	1.21
pH*no. of exposures	0.82	0.62	0.37	0.82	1.36
Replicate leaves	0.95	0.00	0.24	0.14	0.68

Table 33a. Analyses of variance of the effect of rain pH, and of repeated exposures, on cell height of various leaf tissues of Alaskan Artemisia.
* = P<0.05 ** = P<0.01 *** = P<0.001

Source	F Values for cell height				
	Upper epidermis	Palisade mesophyll	Upper spongy mesophyll	Lower spongy mesophyll	Lower epidermis
Rain pH	14.60***	10.18***	2.06	1.31	4.28*
No. of exposures	7.52***	3.62*	0.14	5.06**	1.03
pH*no. of exposures	1.91	1.92	0.42	0.41	1.50
Replicate leaves	1.21	0.35	0.12	0.44	0.39

Table 33b. Analyses of variance of the effect of rain pH, and of repeated exposures, on cell width of various leaf tissues of Alaskan Artemisia.
* = P<0.05 ** = P<0.01 *** = P<0.001

Source	F Values for cell width				
	Upper epidermis	Palisade mesophyll	Upper spongy mesophyll	Lower spongy mesophyll	Lower epidermis
Rain pH	9.17***	6.58**	2.25	0.74	4.45*
No. of exposures	5.25**	1.02	0.18	0.65	1.21
pH*no. of exposures	0.82	0.62	0.37	0.82	1.36
Replicate leaves	0.95	0.00	0.24	0.14	0.68

Table 34. Duncan's Multiple Range tests to examine the trends of repeated rain exposures.

$\bar{x}$ cell size (μm) (means with the same letter are not significantly different, $\alpha = 0.05$)				
Palisade mesophyll (2) height (Smoking Hills)	A 55.94 4 exposures	AB 47.32 8 exposures	BC 40.69 2 exposures	C 33.59 1 exposure
Upper epidermis height (Alaska)	A 15.63 8 exposures	AB 13.89 2 exposures	BC 9.95 1 exposure	C 6.94 4 exposures
Upper epidermis width (Alaska)	A 21.93 8 exposures	A 21.14 2 exposures	A 16.61 1 exposure	B 8.63 4 exposures
Palisade mesophyll height (Alaska)	A 44.87 8 exposures	A 42.55 2 exposures	AB 35.00 1 exposure	B 27.71 4 exposures
Lower spongy mesophyll height (Alaska)	A 25.90 8 exposures	B 16.55 2 exposures	B 14.92 4 exposures	B 14.38 1 exposure

NOTE: All other tissues for the two populations were non-significant (refer to Tables 32a, b and 33a, b) using ANOVA.

Table 35. Analyses of variance (ANOVA) of cell height and width of various leaf tissues from two clones of *Artemisia tilesii* (Smoking Hills clone 11 and Alaska clone 3).
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

	F values for effect of rain pH on cell size	
	Smoking Hills (SH 11)	Alaska (AL 3)
Cell height:		
upper epidermis	34.41***	9.15***
palisade (1) mesophyll	31.00***	7.72**
palisade (2) mesophyll	7.49**	not present
upper spongy mesophyll	not present	2.40
lower spongy mesophyll	0.58	1.09
lower epidermis	1.60	3.99*
Cell width:		
upper epidermis	27.52***	7.17**
palisade (1) mesophyll	2.48	6.95**
palisade (2) mesophyll	3.21	not present
upper spongy mesophyll	not present	2.64
lower spongy mesophyll	1.33	0.78
lower epidermis	5.56**	4.18*

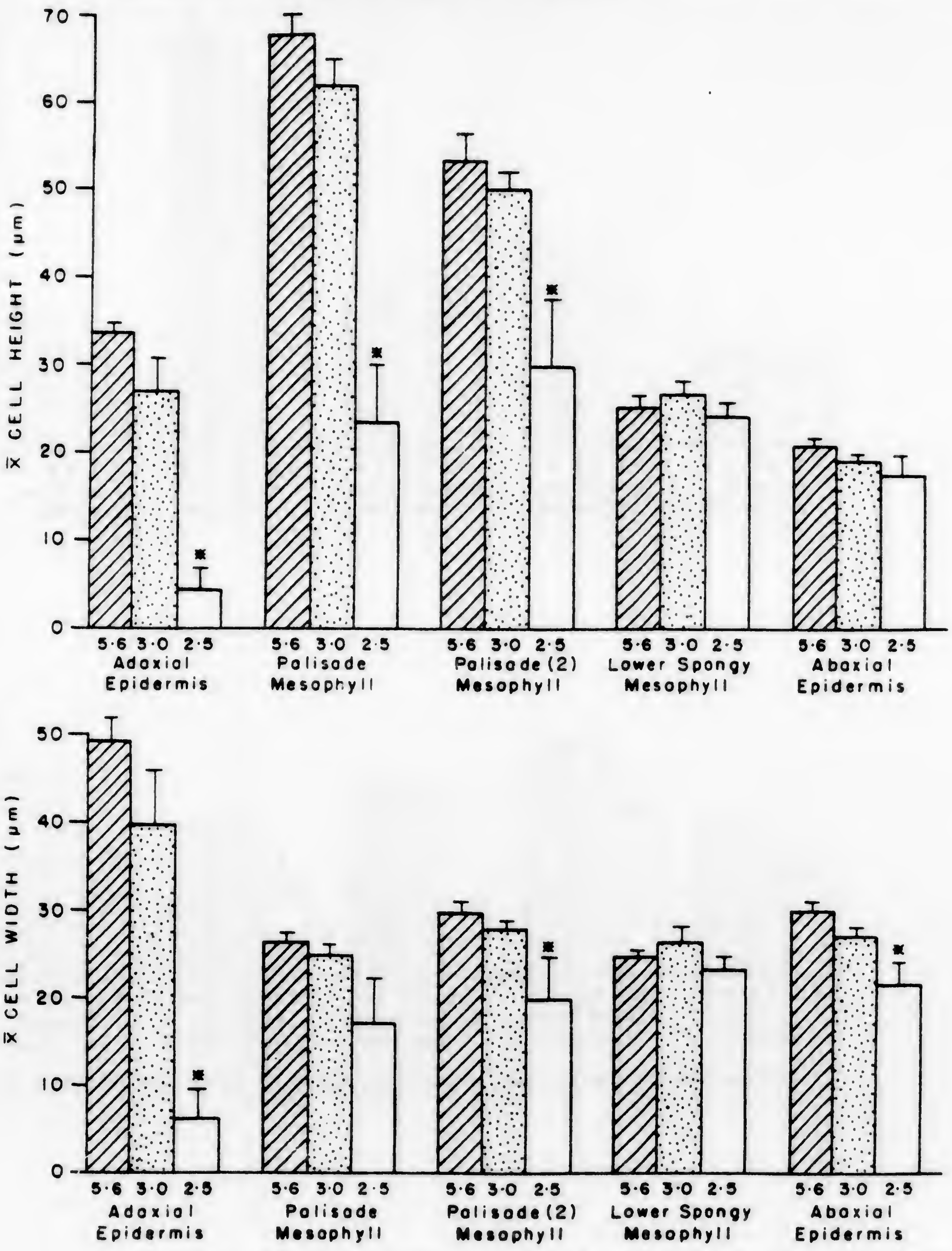
NOTE: ANOVA carried out on the combined data from 1, 2, 4, and 8 rainfall exposures.

Table 36. Measurements of lesion width and depth, according to the severity of cellular injury.
(Total number of lesions was 52 and 241 respectively, for Smoking Hills and Alaskan leaves.)

Pop'n	Measurement (μm)	Lesion category[a]							
		No damage		Minor		Moderate		Severe	
		$\bar{x}$	(SE)	$\bar{x}$	(SE)	$\bar{x}$	(SE)	$\bar{x}$	(SE)
Alaska (AL 3)	Lesion width	0	(0)	56.6	(8.4)	357.4	(112.0)	824.0	(159.0)
	Lesion thickness	0	(0)	9.5	(1.6)	113.1	(15.3)	130.0	(17.5)
Smoking Hills (SH 11)	Lesion width	0	(0)	148.1	(67.4)	617.6	(118.7)	569.6	(60.9)
	Lesion thickness	0	(0)	24.4	(5.3)	279.7	(62.8)	340.1	(47.9)

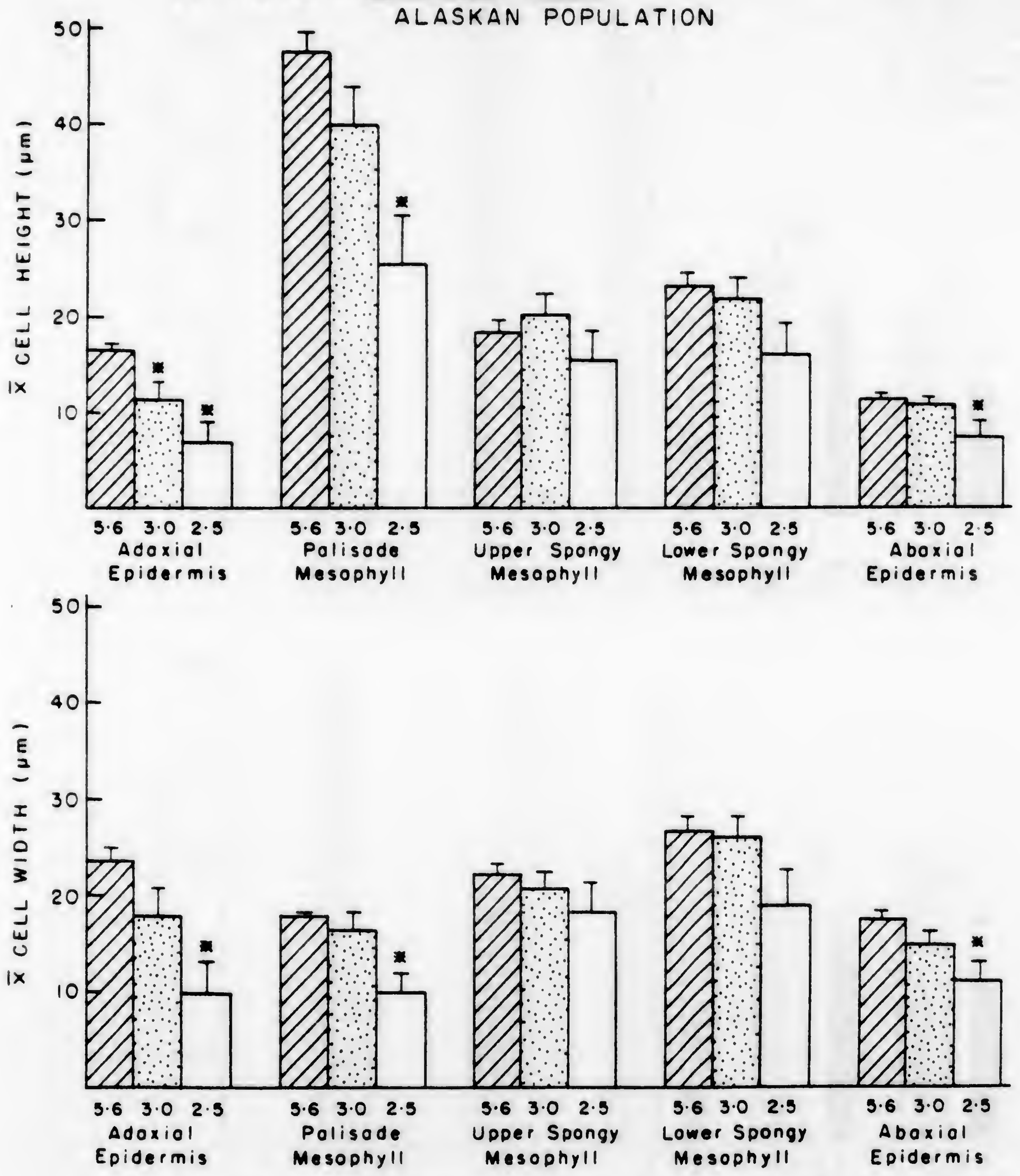
[a] Injury symptoms used to categorize lesions are given in Table 11.

Figure 10
The effect of simulated acid rain on cell dimensions in leaf tissues of *Artemisia tilesii*: Smoking Hills population.



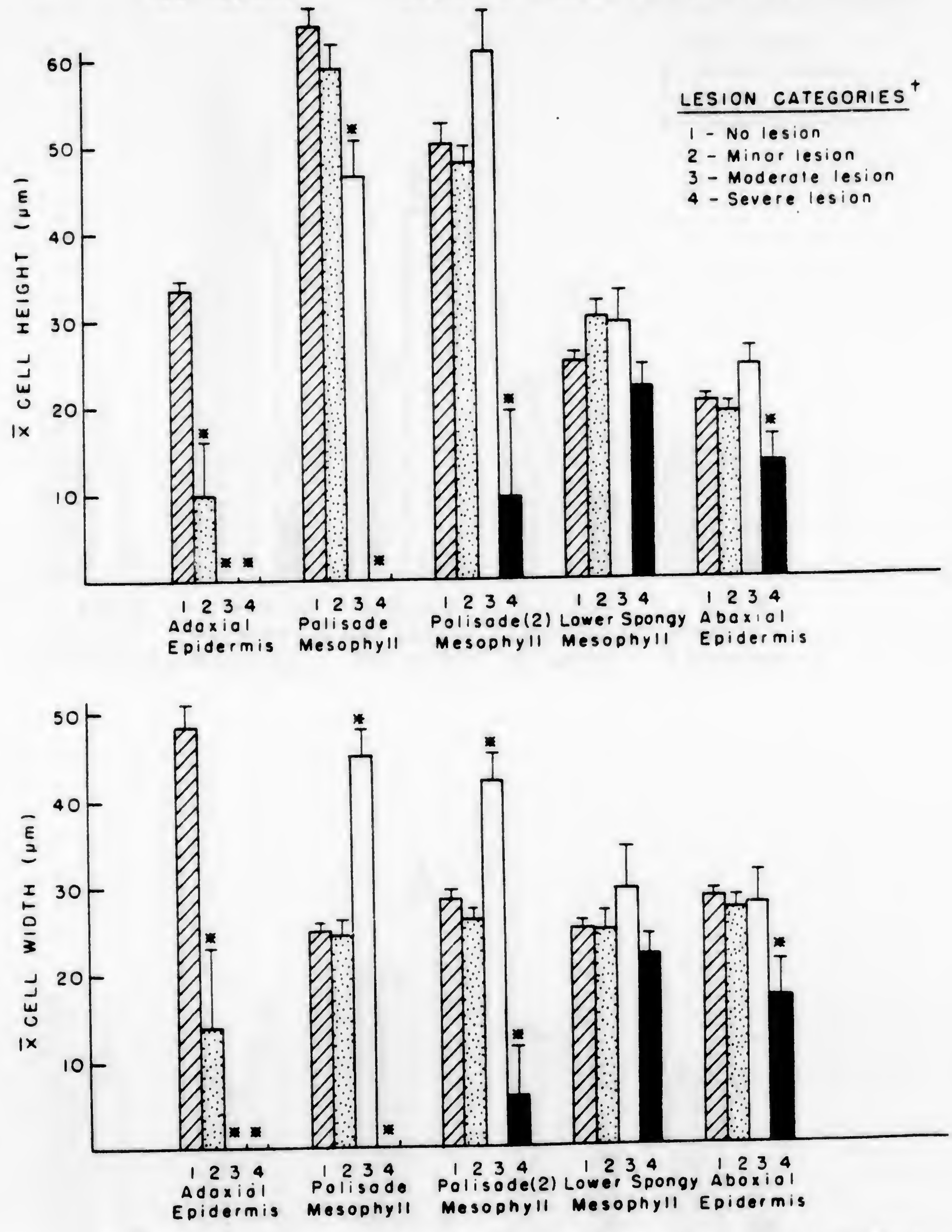
* = $p < 0.05$ Test of significance used was a Duncan's Multiple Range Test.

Figure 11 The effect of simulated acid rain on cell dimensions in leaf tissues of *Artemisia tilesii*.



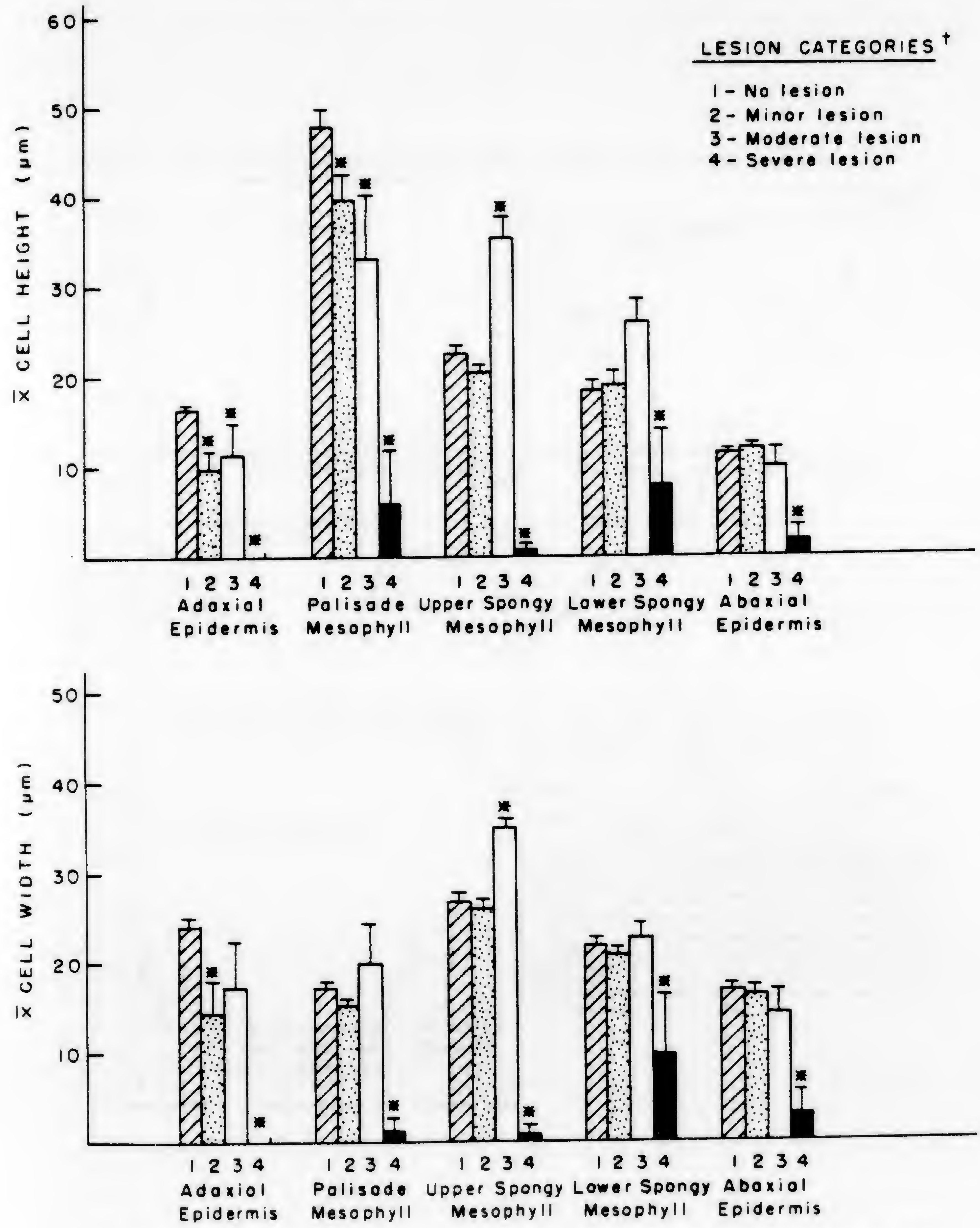
* = $P < 0.05$ Test of significance used was a Duncan's Multiple Range Test.

Figure 12 The effect of simulated acid rain on cell dimensions in various leaf lesions. SMOKING HILLS POPULATION



* = $P<0.05$ Test of significance used was a Duncan's Multiple Range Test.
† = Injury symptoms used to categorize lesions are given in Table II.

Figure 13 The effect of simulated acid rain on cell dimensions in various leaf lesions. ALASKAN POPULATION



* = $P<0.05$ Test of significance used was a Duncan's Multiple Range Test.

† = Injury symptoms used to categorize lesions are given in Table II.

Figure 14 (a–e) Changes occurring in leaf tissues across a severe lesion (pH 3·0, Smoking Hills, clone 11).

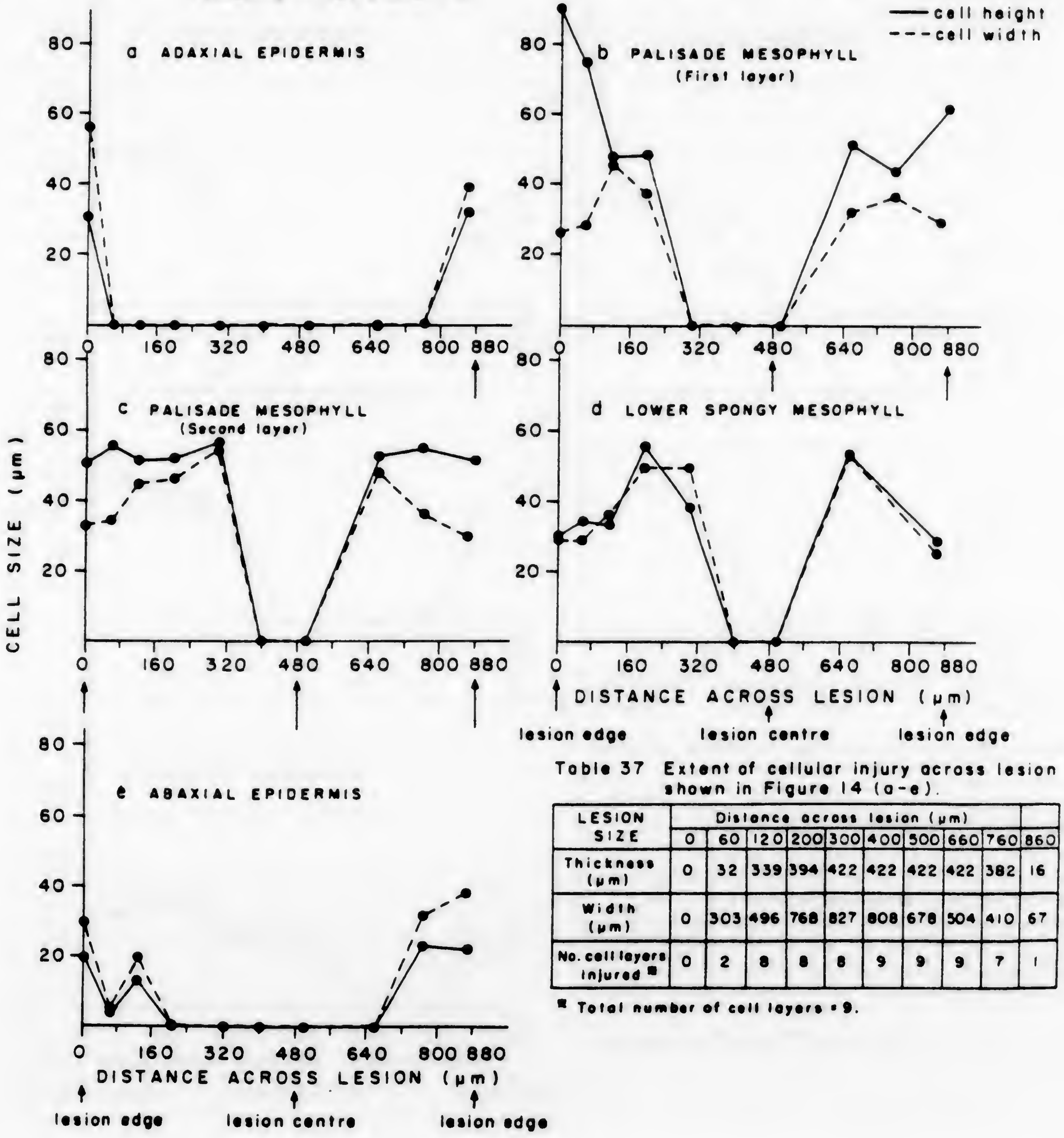


Table 37 Extent of cellular injury across lesion shown in Figure 14 (a–e).

LESION SIZE	Distance across lesion (µm)									
	0	60	120	200	300	400	500	660	760	860
Thickness (µm)	0	32	339	394	422	422	422	422	382	16
Width (µm)	0	303	496	768	827	808	678	504	410	67
No. cell layers injured ■	0	2	8	8	8	9	9	9	7	1

■ Total number of cell layers = 9.

Figure 15 (a–e) Changes occurring in leaf tissues across a severe lesion (pH 2·5, Alaska, clone 3).

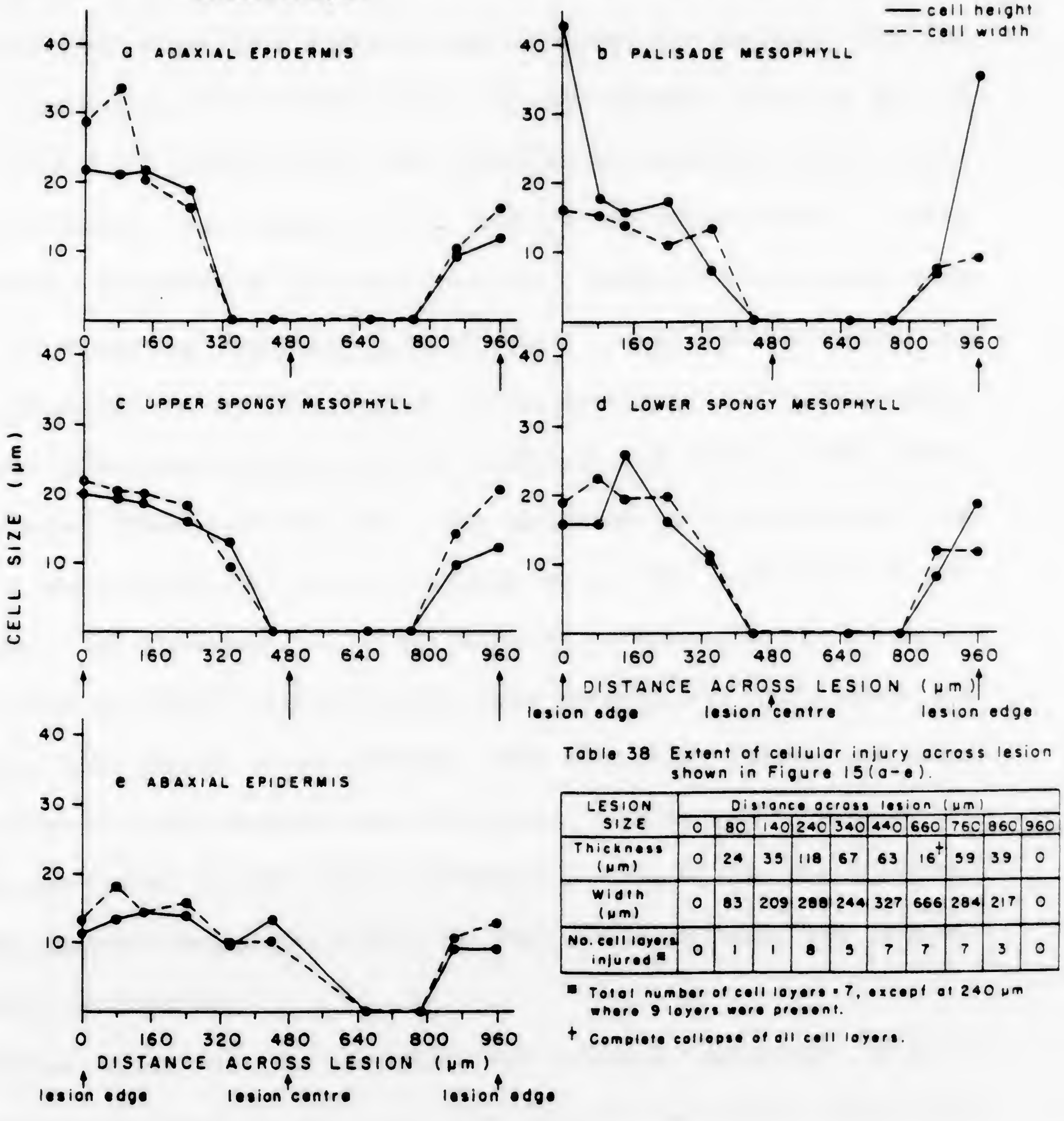


Table 38 Extent of cellular injury across lesion shown in Figure 15(a–e).

LESION SIZE	Distance across lesion (μm)									
	0	80	140	240	340	440	660	760	860	960
Thickness (μm)	0	24	35	118	67	63	16†	59	39	0
Width (μm)	0	83	209	288	244	327	666	284	217	0
No. cell layers injured ■	0	1	1	8	5	7	7	7	3	0

■ Total number of cell layers = 7, except at 240 μm where 9 layers were present.

† Complete collapse of all cell layers.

d. Three-dimensional nature of lesions

Up to this point, lesion development has been considered as occurring in a vertical plane from the adaxial epidermis toward the interior of the leaf. The development of lesions is actually more complex and should be viewed in a three-dimensional framework. Examination of sequential sections taken through two lesions - Smoking Hills, clone 11, pH 3.0 (Plate 42a - h) and Alaska, clone 3, pH 2.5 (Plate 43a - f) demonstrates the variation in structure that occurs across a lesion. The changes in the severity of injury across a lesion are further examined by plotting mean cell sizes of each tissue type taken at intervals across the two lesions. Both lesions reveal an abrupt increase in cellular injury at the centre of the lesion, where all cell layers are injured and the thickness and width of the lesion are greatest (Tables 37 and 38). The thickness of the lesion for the Alaskan plant (Table 38) has one exception to this generality at 660 µm, where the thickness is 16 µm compared to approximately 60 µm on either side of this. Plate 43 shows that although all cell layers are damaged, the damage is so drastic that the leaf tissue collapses completely to a very narrow band of tissue. Towards either edge of the lesion the number of cell layers affected is reduced (in the direction of the adaxial epidermis) until at the periphery only the adaxial epidermis is injured.

While cells of the adaxial and abaxial epidermis show a progressive decrease in mean cell size towards the lesion centre, the mesophyll tissues reveal quite a different response (Figs. 14a - e and 15a - e). In sections through one lesion (Smoking Hills, clone 11) cell hypertrophy, evident by the increase in cell width (palisade mesophyll, both layers) and cell width and height (lower spongy mesophyll), occurs in the area adjacent to the collapsed tissue at the lesion centre (Fig. 14b - d). Micrographs taken of sections in this area (Plate 42d and e) show that this results in reduced intercellular space in this band of tissue separating the severely damaged centre of the lesion from undamaged tissue on either side.

No cell enlargement occurred in the mesophyll for the other lesion (Alaska, clone 3). The effect of acid injury here was a progressive decrease in mean cell size of palisade and spongy mesophyll towards the centre of the lesion (Fig. 15b - d). Micrographs of sequential sections through this lesion do, however, reveal that intercellular space is drastically reduced on either side of a narrow band of severely injured tissue (Plate 43d, e) due to cell hyperplasia. The presence of new cross-walls between cells, and the obvious nuclei in the newly divided cells, provide evidence of recent cell divisions.

e. Effect of rainfall pH and repeated application of acidic rain on lesion development

In general, more advanced stages in lesion development were represented in sectioned tissue from the more acidic (pH 2.5) treatment and only early stages in lesion development in sectioned tissue for pH 3.0 (Table 39). Thus, 44% of lesions produced by rainfall with a pH of 2.5 were stage IV lesions with leaf tissue completely collapsed, while only 6% of lesions from the pH 3.0 treatment were at this advanced stage. In approximately half of the sectioned leaf tissue from the pH 3.0 treatment no cellular injury was observed, whereas 87% of all leaves sectioned from the pH 2.5 treatment contained a lesion.

It was expected that the sequence of stages in lesion development evident in leaves exposed to increasingly acidic rainfall might be similar to that which would subsequently occur if lesions were examined with each additional exposure at pH 3.0 or 2.5. That is, if lesions were sampled sequentially with each exposure a shift from lesions of early developmental stages to advanced stages would occur. This, however, was not the case, since lesions in various stages of advancement were seen after but one 20 minute exposure to acid rain (pH 2.5 and 3.0) (Table 2). More importantly, there was no consistent effect of repeated spraying on cell dimensions (which is a measure of the amount of cell injury) for five cell types of the two clones. Although analyses of variance performed on the cell height and width of injured and non-injured leaves were significantly ($p<0.05$; Table 32 and 33) related to number of sprayings in 5 of the 20 tests performed, further examination using Duncan's Multiple Range Test showed that the pattern of variation was not consistent with the number of sprayings (Table 34). A restriction on the lesion size (<100 µm diameter) of embedded lesions for plastic sectioning probably resulted in the apparent lack of effect of repeated exposure on lesion severity. A shift towards larger lesions was observed with cleared leaves after repeated exposures. Treatment of the cross sectional cell size data (Figs. 10 - 13) pooled the leaves which were sampled from the 4 rainfall exposures, within each pH treatment.

Table 39. The frequency of lesions in various stages of development.

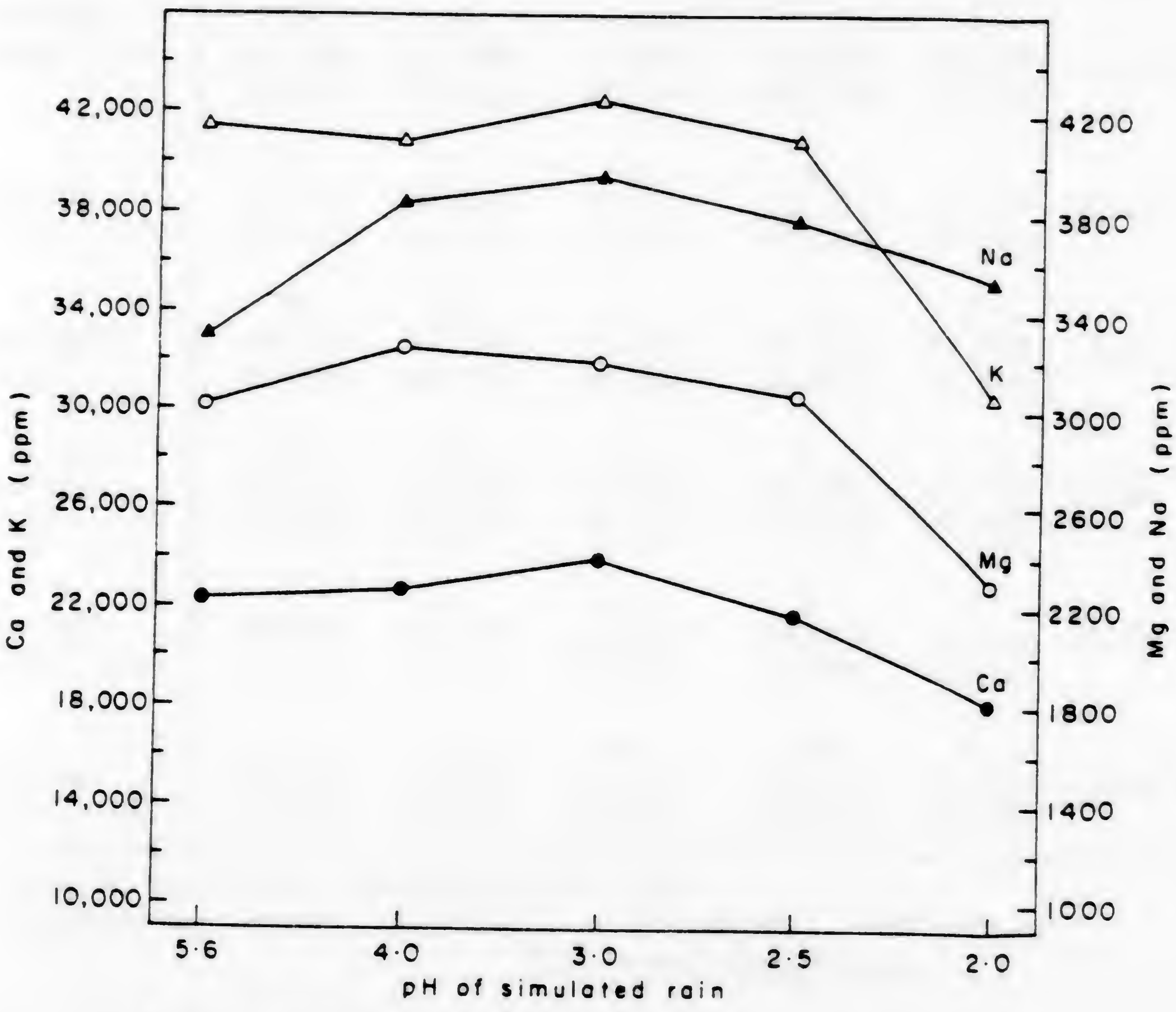
pH and Population	Number of Sprayings (20 min)	Frequency of lesion type (total scored=4)[a]			
		No injury	Minor-Stage 1	Moderate-Stages 2&3	Severe-Stage 4
2.5					
Smoking Hills	1	1			3
	2	1	1		2
	4		1	2	1
	8			2	2
Alaska	1		3		1
	2	2	1		1
	4			2	2
	8		1	1	2
Total (%)		13%	22%	22%	44%
3.0					
Smoking Hills	1	3	1		
	2	4			
	4	2	1		1
	8	3	1		
Alaska	1	1	2	1	
	2	2	2		
	4	2	1		1
	8	1	2	1	
Total (%)		56%	31%	6%	6%

[a] Injury symptoms used to categorize lesions are given in Table 11.

Effect of simulated acid rain on cation loss from leaves

Foliage of six *Artemisia* clones was chemically analyzed to determine cation concentrations, in order to find whether leaching from leaves occurred in response to acidic "rainfalls". Plants were given 12 six minute exposures to simulated acid rain over a period of 4 weeks. Figure 16 and Tables 40 - 43 show the reduction of most of the measured cations in the foliage as a result of low pH treatment. Significant losses in foliar calcium, magnesium and potassium were observed for the pH 2.0 treatment compared with the control, although no effect was apparent at the pH treatments above 2.0, using Duncans's Multiple Range Tests (Tables 40 - 42). Foliar sodium concentrations increased with increasing rain acidity, though these increases were not statistically significant.

Figure 16 Concentration of cations remaining in *Artemisia tilesii* leaves† exposed to simulated acid rain.■



■Plants were given 12 six minute 'rain' exposures over a four week period.

†Concentrations are the mean values of 6 Smoking Hills clones.

Table 40. Concentration of calcium remaining in Artemisia leaves[a] of six Smoking Hills clones exposed to simulated acid rain. (Plants were exposed to 12 six minute 'rainfalls' over four weeks.)

Clone	Simulated rain pH					
	5.6	4.0	3.0	2.5	2.0	F Values[b]
	ppm Ca					
Smoking Hills 11	A[c] 22,237 ±2,760	A 22,949 ±3,410	A 21,830 ±3,104	A 20,653 ±2,571	A 20,289 ±2,347	1.73 N.S.
Smoking Hills 9	A 25,272 ±2,122	A 23,674 ±1,270	A 26,217 ±1,315	A 25,461 ±2,042	B 19,253 ±1,564	10.82 ***
Smoking Hills 5	AB 23,541 ±1,010	AB 23,880 ±3,174	A 25,854 ±1,263	B 22,351 ±2,663	C 17,786 ±1,596	8.13 **
Smoking Hills 3	AB 25,301 ± 974	AB 24,634 ±1,275	A 26,181 ±1,174	B 22,805 ± 331	C 17,778 ±1,786	12.25 ***
Smoking Hills 8	A 20,091 ±2,056	A 19,476 ±1,123	A 21,337 ±2,562	A 20,808 ±2,979	A 19,730 ±3,731	0.34 N.S.
Smoking Hills 12	A 18,333 ± 866	A 21,280 ±3,953	A 22,148 ±3,410	AB 18,152 ±1,156	B 14,356 ±1,694	5.86 **

[a] Mean of four plant replication ± 1 S.D.

[b] Significance level: * $p<0.05$ ** $p<0.01$ *** $p<0.001$
N.S. = not significant

[c] Means with the same letter do not differ significantly (α=0.05, Duncan's Multiple Range Test).

Table 41. Concentration of magnesium remaining in Artemisia leaves[a] of six Smoking Hills clones exposed to simulated acid rain. (Plants were exposed to 12 six minute 'rainfalls' over four weeks.)

Clone	Simulated rain pH					
	5.6	4.0	3.0	2.5	2.0	F Values[b]
	ppm Mg					
Smoking Hills 11	A[c] 2,563 ± 127	A 2,920 ± 314	A 2,715 ± 594	A 2,531 ± 193	B 1,993 ± 331	3.85 *
Smoking Hills 9	A 3,644 ± 179	A 3,259 ± 246	A 3,520 ± 73	A 3,365 ± 155	B 2,276 ± 315	23.62 ***
Smoking Hills 5	A 2,823 ± 114	A 3,069 ± 310	A 2,939 ± 199	A 2,884 ± 173	B 1,874 ± 51	25.45 ***
Smoking Hills 3	A 3,238 ± 126	A 3,589 ± 460	A 3,439 ± 153	A 3,344 ± 118	B 2,119 ± 290	19.81 ***
Smoking Hills 8	A 2,964 ± 336	A 3,250 ± 518	A 3,492 ± 274	A 3,281 ± 557	A 3,040 ± 331	1.67 N.S.
Smoking Hills 12	AB 2,943 ± 344	A 3,426 ± 433	AB 3,057 ± 217	AB 2,957 ± 178	B 2,436 ± 172	1.78 N.S.

[a] Mean of four plant replication ± 1 S.D.

[b] Significance level: * $p<0.05$ ** $p<0.01$ *** $p<0.001$
N.S. = not significant

[c] Means with the same letter do not differ significantly ($\alpha=0.05$, Duncan's Multiple Range Test).

Table 42. Concentration of potassium remaining in Artemisia leaves[a] of six Smoking Hills clones exposed to simulated acid rain. (Plants were exposed to 12 six minute 'rainfalls' over four weeks.)

Clone	Simulated rain pH					
	5.6	4.0	3.0	2.5	2.0	F Values[b]
			ppm K			
Smoking Hills 11	AB[c] 35,097 ±2,176	A 39,670 ±2,916	AB 36,207 ±4,567	B 34,551 ±1,713	C 22,633 ±2,302	19.73 ***
Smoking Hills 9	A 44,692 ±6,986	AB 42,748 ±3,940	A 45,696 ±4,662	A 43,745 ±1,711	B 35,364 ±7,057	2.43 N.S.
Smoking Hills 5	AB 37,735 ±3,071	B 37,031 ±3,440	A 42,278 ±5,452	A 44,937 ±6,890	C 29,086 ±3,774	6.51 **
Smoking Hills 3	B 46,109 ±3,134	B 46,501 ±3,133	A 53,550 ±2,601	B 48,343 ±2,043	C 37,055 ±2,932	18.16 ***
Smoking Hills 8	A 43,641 ±3,329	AB 37,180 ±5,476	A 38,518 ±5,564	B 31,518 ±1,493	B 31,509 ±4,136	5.89 **
Smoking Hills 12	A 41,623 ±5,782	A 42,530 ±6,664	A 38,804 ±3,277	A 42,708 ±5,955	B 27,503 ±1,441	6.53 **

[a] Mean of four plant replication ± 1 S.D.

[b] Significance level: * $p<0.05$ ** $p<0.01$ *** $p<0.001$
N.S. = not significant

[c] Means with the same letter do not differ significantly ($\alpha=0.05$, Duncan's Multiple Range Test).

Table 43. Concentration of sodium remaining in Artemisia leaves[a] of six Smoking Hills clones exposed to simulated acid rain. (Plants were exposed to 12 six minute 'rainfalls' over four weeks.)

Clone	Simulated rain pH					
	5.6	4.0	3.0	2.5	2.0	F Values[b]
			ppm Na			
Smoking Hills 11	AB[c] 3,628 ± 933	AB 3,828 ± 515	AB 3,802 ± 585	A 5,602 ±2,628	B 3,165 ±1,040	1.83 N.S.
Smoking Hills 9	A 2,380 ± 783	A 2,133 ± 159	A 2,165 ± 525	A 1,865 ± 413	A 2,285 ± 712	0.48 N.S.
Smoking Hills 5	A 4,741 ± 901	A 3,992 ±1,046	A 4,782 ± 962	A 3,809 ± 310	A 5,229 ±1,267	1.49 N.S.
Smoking Hills 3	B 1,867 ± 491	A 2,639 ± 540	AB 2,412 ± 486	AB 1,944 ± 384	B 1,792 ± 413	2.56 N.S.
Smoking Hills 8	A 4,412 ± 708	A 7,069 ±2,381	A 6,693 ± 961	A 6,399 ±2,258	A 5,312 ±1,635	1.69 N.S.
Smoking Hills 12	A 2,810 ± 587	A 3,377 ±1,117	A 3,811 ± 771	A 2,972 ± 765	A 3,424 ± 530	1.20 N.S.

[a] Mean of four plant replication ± 1 S.D.

[b] Significance level: * $p<0.05$ ** $p<0.01$ *** $p<0.001$
N.S. = not significant

[c] Means with the same letter do not differ significantly ($\alpha=0.05$, Duncan's Multiple Range Test).

Effect of simulated acid rain on plant growth

A significant reduction in root weight was observed as a result of the pH 2.0 treatment (Fig. 17, Table 44). Plants receiving pH 2.0 rainfall for a period of 4 weeks incurred a 60% reduction in root dry weight, while the pH 2.5, 3.0, and 4.0 treatments showed no reductions when compared to the control. Plastic lids, covering the pots in which plants were grown, precluded the entry of any significant quantity of rainfall solutions and measurements of the soil pH showed that it was not lowered as a result of the rainfall treatments (Table 45). The observed reduction in root weight is, therefore, attributed to a mechanism other than the direct effect of acid on the root system.

The dry weight of shoots in contrast, was unaffected by the pH treatments. Individual analyses of variance for the shoot weight of six clones indicated a significant acid effect occurred for only one clone (clone 12)(Table 44).

The number of leaves which were produced on these plants showed a similar trend to that of shoot weight, with no significant effect of acid treatment (Table 46). Although two of the clones had a significant F statistic across the pH gradient for the number of leaves produced, examination of the data indicated that the number of leaves varied randomly rather than in a sequence across the pH gradient.

Since significant effects on root weight were produced at low pH over a relatively short term rainfall treatment, it was of interest to determine the effects of long term exposure to acidic rain on plant growth. Few studies to date have examined acid rain effects on plant growth over the length of the growing season. The highest acidity of the rainfall treatments used in this experiment was pH 2.5, since this

Figure 17 Dry weight reduction in 6 Smoking Hills clones exposed to simulated acid rain. (Plants harvested after 4 weeks exposure.)

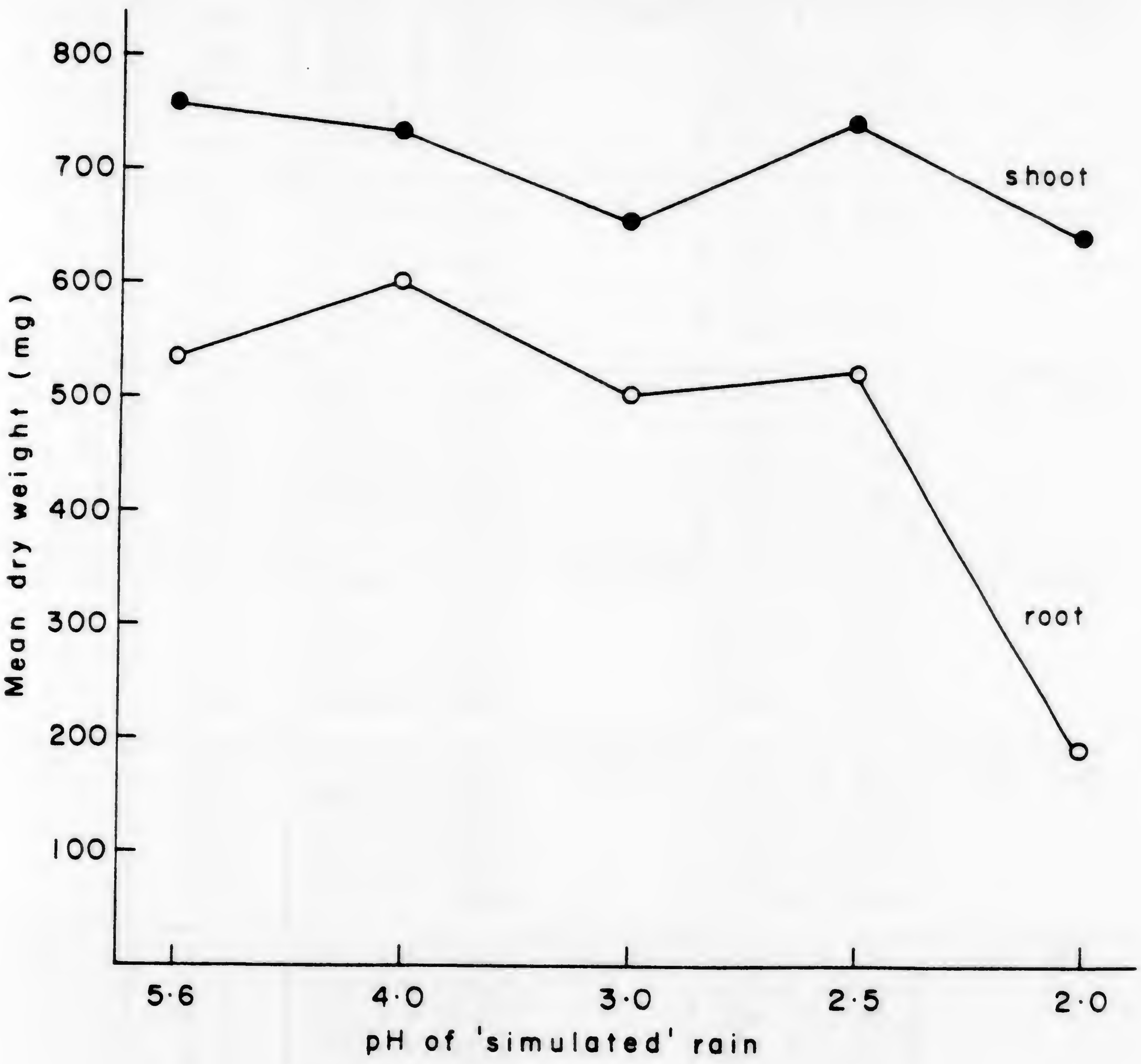


Table 44. Dry weight results for control (pH 5.6) and acid-treated Smoking Hills clones of *Artemisia tilesii*.

Clone (Smoking Hills)	Simulated rain pH[b]	Dry weight (mg)[a]			
		Root Weight	F Value[c]	Shoot Weight	F Value[c]
3	5.6	738.7 ± 287.4	30.5***[d]	1160.9 ± 203.9	1.0 N.S.
	4.0	903.1 ± 309.7		1087.1 ± 122.2	
	3.0	689.1 ± 167.8		935.8 ± 199.5	
	2.5	765.9 ± 221.0		1015.9 ± 340.4	
	2.0	223.9 ± 62.5		976.0 ± 330.9	
5	5.6	621.6 ± 395.1	16.7 ***	576.9 ± 229.2	1.2 N.S.
	4.0	579.1 ± 261.7		589.4 ± 129.1	
	3.0	575.2 ± 349.7		542.2 ± 219.8	
	2.5	529.2 ± 238.0		629.4 ± 234.3	
	2.0	138.7 ± 105.0		463.6 ± 181.9	
8	5.6	505.9 ± 155.9	7.4 ***	589.7 ± 133.2	1.4 N.S.
	4.0	372.8 ± 195.0		459.0 ± 214.0	
	3.0	225.6 ± 118.7		368.1 ± 160.2	
	2.5	248.9 ± 127.4		426.1 ± 156.2	
	2.0	159.3 ± 155.7		494.1 ± 253.8	
9	5.6	639.9 ± 270.8	13.5 ***	954.4 ± 345.8	1.5 N.S.
	4.0	994.2 ± 322.2		1012.6 ± 263.9	
	3.0	910.6 ± 245.4		1001.9 ± 152.6	
	2.5	992.9 ± 298.6		1233.1 ± 196.1	
	2.0	368.8 ± 149.9		1018.4 ± 175.0	
11	5.6	365.2 ± 181.6	5.7 **	640.1 ± 201.0	0.6 N.S.
	4.0	388.8 ± 136.3		623.9 ± 159.1	
	3.0	327.8 ± 147.1		593.7 ± 129.9	
	2.5	265.5 ± 138.7		557.0 ± 187.5	
	2.0	147.2 ± 46.6		527.0 ± 122.3	
12	5.6	337.3 ± 143.6	10.1 **	649.9 ± 149.0	4.0 **
	4.0	368.9 ± 186.0		630.0 ± 240.6	
	3.0	282.8 ± 116.7		489.1 ± 137.8	
	2.5	322.0 ± 148.9		586.2 ± 217.4	
	2.0	118.9 ± 47.0		372.9 ± 186.2	

[a] Mean of 10 plant replication ± 1 S.D.

[b] Plants were given 12 six minute exposures over a 4 week period

[c] F values after applying a log transformation to the dry weight values

[d] Significance level: *$P<0.05$ **$P<0.01$ ***$P<0.001$

N.S. = not significant

Table 45. Soil pH of pots before and after rain simulation experiments. (Values are the mean of 5 pot replication)

pH of rainfall applied	Initial soil pH	Final soil pH
5.6	7.9	7.8
4.0	7.9	7.9
3.0	7.9	8.0
2.5	7.9	7.9
2.0	7.9	7.9

[a] pH of soil in pots was measured after 4 weeks. Plastic lids with openings in the centre for plants to grow through were used to prevent solutions from entering soil. Plants were watered with tap water from trays below.

was considered to be below the minimum pH of rain presently occurring in North America.

The results of individual analyses of variance of the dry weight of roots and shoots of 6 clones used in this experiment, from both Smoking Hills and Alaskan populations, indicated no significant effect of rainfall acidity on either of these growth parameters for plants harvested after 6 weeks of treatment (Table 47). Similarly, the number of leaves produced was not significantly affected by the pH treatments (Table 48). Although not significant, there does appear however to be a stimulation in the dry weight of both roots and shoots at intermediate pH levels (pH 3.5 and 3.0), followed by a decrease in root and shoot weight at pH 2.5, for all but one of the six clones from the two populations (Figs. 18a - c, 19a - c). The lack of significance of this common trend amongst clones may be the result of the small sample size (n=7), for each of the 6 clones. Unfortunately, the large variation in the weights of individual clones meant that the variation within pH treatments was too large to allow examination of the differences across the pH treatment, if the mean weight of all clones from each population (n = 21) was considered (Fig. 20). Plants which received the pH treatments for a period of 12 weeks also showed an insignificant acid effect on root and shoot weights (ANOVA $P<0.05$). The data for 12 weeks illustrated much the same trend as that of dry weight measured at 6 weeks. Although non-significant, ($P<0.05$), stimulations of root weight and shoot weight may occur at pH 3.5 and 3.0 compared to the control (pH 5.6), and at pH 2.5 the weights are very similar to the control treatment weights (Figs. 18d - f, 19d - f).

Table 46. Number of new leaves produced on Artemisia plants exposed to simulated acid rain - Experiment 1[a].

Clone number	Simulated rain pH					
	5.6	4.0	3.0	2.5	2.0	F Values[c]
	Number of leaves produced[b]					
Smoking Hills 11	15.7 ±2.1	15.8 ±2.8	15.2 ±2.3	13.8 ±1.0	14.7 ±2.4	1.26 N.S.
Smoking Hills 9	11.5 ±2.7	9.0 ±3.4	9.6 ±3.0	10.5 ±2.8	14.2 ±2.2	4.47 **
Smoking Hills 5	15.6 ±5.3	18.6 ±6.7	15.6 ±6.1	17.5 ±3.5	17.8 ±2.4	0.86 N.S.
Smoking Hills 3	12.2 ±3.6	11.5 ±3.6	11.3 ±3.4	12.1 ±3.7	15.6 ±2.0	3.69 *
Smoking Hills 8	12.8 ±2.0	16.3 ±2.0	15.0 ±2.5	12.1 ±3.4	14.0 ±2.9	3.69 *
Smoking Hills 12	20.7 ±3.9	19.9 ±5.0	17.5 ±5.1	18.0 ±5.0	16.6 ±3.7	0.91 N.S.

[a] Values are the number of new leaves produced in 4 weeks prior to harvest. Plants received a total of 12 six minute exposures to simulated rain over a 4 week period.

[b] Mean of 10 plant replication ± 1 S.D.

[c] F values after applying a log transformation to the data for the number of leaves produced

Significance level: $*P<0.05$ $**P<0.01$ $***P<0.001$
N.S. = not significant

Table 47. Dry weight results for control (pH 5.6) and acid-treated Artemisia clones from two populations.

Pop'n and clone	Rain pH	ROOT WEIGHT (MG)[a]		SHOOT WEIGHT (MG)[a]	
		6 wk harvest[b] $\bar{x}$ ± S.D.	12 wk harvest $\bar{x}$ ± S.D.	6 wk harvest $\bar{x}$ ± S.D.	12 wk harvest $\bar{x}$ ± S.D.
Smoking Hills 8	5.6	365.6 ± 95.9	522.5 ± 156.4	351.6 ± 70.3	601.0 ± 272.5
	4.0	386.9 ± 150.9	465.0 ± 103.9	365.5 ± 95.5	441.6 ± 98.1
	3.5	416.5 ± 246.7	506.2 ± 117.1	498.4 ± 267.6	596.2 ± 151.5
	3.0	571.1 ± 281.4	448.6 ± 157.6	499.2 ± 252.2	564.9 ± 239.9
	2.5	350.4 ± 132.1	431.9 ± 147.4	402.8 ± 170.5	592.8 ± 289.7
F Value		1.40 N.S.[d]	0.63 N.S.	0.90 N.S.	0.48 N.S.
Smoking Hills 9	5.6	500.2 ± 163.4	581.4 ± 135.2	578.2 ± 185.8	692.0 ± 93.4
	4.0	527.7 ± 128.4	503.5 ± 272.3	591.2 ± 133.0	582.6 ± 307.7
	3.5	527.2 ± 150.5	595.8 ± 136.3	583.3 ± 136.5	663.7 ± 181.8
	3.0	546.1 ± 208.8	657.5 ± 136.3	553.8 ± 202.0	728.0 ± 66.1
	2.5	461.6 ± 136.3	543.0 ± 303.3	483.2 ± 143.1	721.2 ± 224.1
F Value		0.30 N.S.	0.68 N.S.	0.52 N.S.	0.90 N.S.
Smoking Hills 11	5.6	383.3 ± 139.6	776.2 ± 148.3	475.6 ± 136.0	726.7 ± 141.6
	4.0	336.9 ± 124.2	504.2 ± 159.3	392.7 ± 152.8	540.2 ± 169.4
	3.5	351.8 ± 136.8	577.4 ± 213.0	419.1 ± 176.1	556.7 ± 178.8
	3.0	328.9 ± 73.9	480.5 ± 217.4	411.5 ± 85.1	423.7 ± 165.7
	2.5	416.1 ± 185.7	547.8 ± 125.8	480.0 ± 149.9	651.4 ± 219.2
F Value		0.27 N.S.	2.73 *	0.51 N.S.	3.42 *

Continued

Table 47 (Continued).

Pop'n and clone	Rain	ROOT WEIGHT (MG)[a]		SHOOT WEIGHT (MG)[a]	
	pH	6 wk harvest[b] $\bar{x}$ ± S.D.	12 wk harvest $\bar{x}$ ± S.D.	6 wk harvest $\bar{x}$ ± S.D.	12 wk harvest $\bar{x}$ ± S.D.
Alaska 2	5.6	423.9 ± 177.5	712.6 ± 347.1	1095.5 ± 420.4	1412.1 ± 241.4
	4.0	450.9 ± 166.1	810.5 ± 313.5	1183.6 ± 237.2	1470.8 ± 411.2
	3.5	535.1 ± 146.0	960.9 ± 318.1	1224.0 ± 248.2	1700.3 ± 439.3
	3.0	424.6 ± 123.8	609.3 ± 212.7	1011.1 ± 244.5	1441.8 ± 549.8
	2.5	409.1 ± 189.4	658.4 ± 360.9	1001.0 ± 325.9	1338.7 ± 693.8
F Value		0.91 N.S.	1.08 N.S.	0.84 N.S.	0.72 N.S.
Alaska 3	5.6	329.4 ± 67.8	440.1 ± 264.4	839.1 ± 196.1	1080.0 ± 465.9
	4.0	309.9 ± 78.3	391.8 ± 57.4	863.7 ± 318.8	998.5 ± 204.5
	3.5	412.8 ± 162.4	436.1 ± 183.7	1122.4 ± 392.1	1222.8 ± 455.7
	3.0	330.3 ± 85.0	558.1 ± 148.8	991.1 ± 286.2	1442.0 ± 387.3
	2.5	330.3 ± 132.8	509.8 ± 177.6	822.7 ± 312.2	1358.8 ± 492.7
F Value		0.53 N.S.	0.83 N.S.	0.99 N.S.	1.30 N.S.
Alaska 5	5.6	595.2 ± 334.5	---	1258.9 ± 638.2	---
	4.0	516.0 ± 261.3	---	1017.0 ± 470.0	---
	3.5	648.9 ± 279.8	---	1162.5 ± 484.8	---
	3.0	562.9 ± 174.0	---	1067.7 ± 328.2	---
	2.5	490.5 ± 267.4	---	1144.1 ± 378.8	---
F Value		0.44 N.S.		0.19 N.S.	

[a] Mean of 7 plant replication.

[b] Total number of rain exposures for plants harvested after 6 weeks and 12 weeks was 12 and 24, respectively.

[c] F values after applying a log transformation to the dry weight values.

Significance level: *P<0.05 **P<0.01 ***P<0.001 N.S.=not significant

NOTE: Anova indicated no significant block effects for the 6 week harvest and significant block effects for Alaska 2 and 3 only, for the 12 week harvest.

Table 48. Number of new leaves produced on Artemisia plants exposed to simulated acid rain - Experiment 2[a].

Clone number	Simulated rain pH					
	5.6	4.0	3.5	3.0	2.5	F Values[c]
	Number of leaves produced[b]					
Smoking Hills 11	13.6 ±4.3	15.1 ±4.4	13.8 ±4.1	15.0 ±3.1	14.3 ±2.1	0.59 N.S.
Smoking Hills 9	19.6 ±1.6	21.2 ±3.8	19.1 ±2.9	19.3 ±2.1	17.4 ±3.5	2.92 *
Smoking Hills 8	13.3 ±6.9	14.1 ±5.5	13.8 ±4.1	12.4 ±2.7	14.3 ±3.6	0.38 N.S.
Alaska 2	11.4 ±1.6	11.1 ±1.9	11.6 ±1.6	12.5 ±2.5	11.3 ±1.7	0.99 N.S.
Alaska 3	18.1 ±5.6	17.4 ±4.6	19.9 ±4.0	20.3 ±3.7	17.9 ±3.0	1.62 N.S.
Alaska 5	14.4 ±3.7	11.9 ±6.8	13.2 ±6.2	13.0 ±3.4	14.7 ±4.2	0.33 N.S.

[a] Values are the number of new leaves produced in 6 weeks prior to harvest. Plants received a total of 12 twenty minute exposures to simulated rain.

[b] Mean of 10 plant replication ± 1 S.D.

[c] F values after applying a log transformation to the data for the number of leaves produced.

Significance level: $*P<0.05$ $**P<0.01$ $***P<0.001$
N.S. = not significant

Figure 18 Effect of rainfall acidity on dry weight of 3 Smoking Hills clones of *Artemisia tilesii* harvested after 6 weeks and 12 weeks. (Each point represents the mean of the weights of 7 plants.)

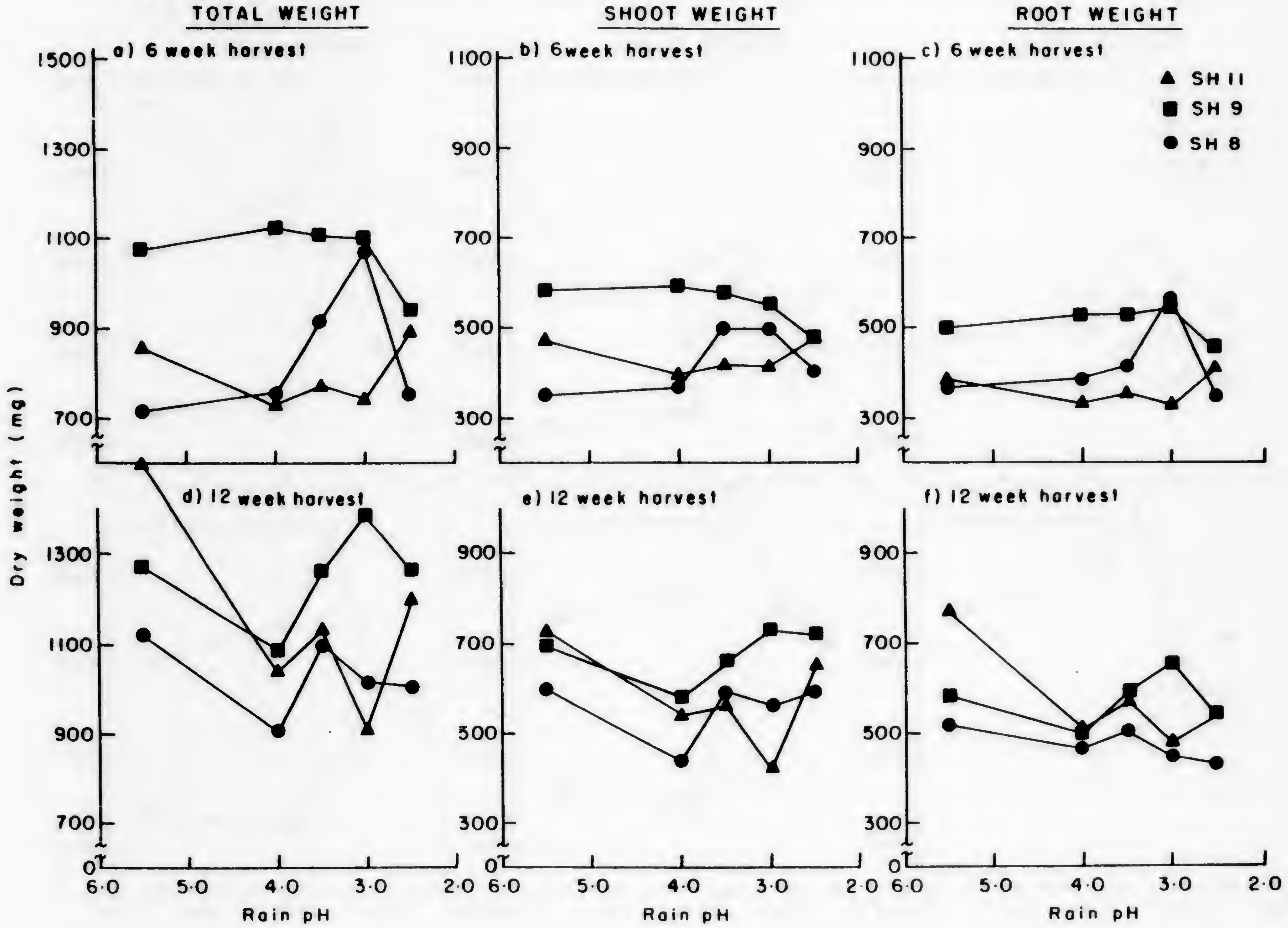


Figure 19 Effect of rainfall acidity on dry weight of 3 Alaskan clones of Artemisia tilesii harvested after 6 weeks and 12 weeks. (Each point represents the mean of the weights of 7 plants.)

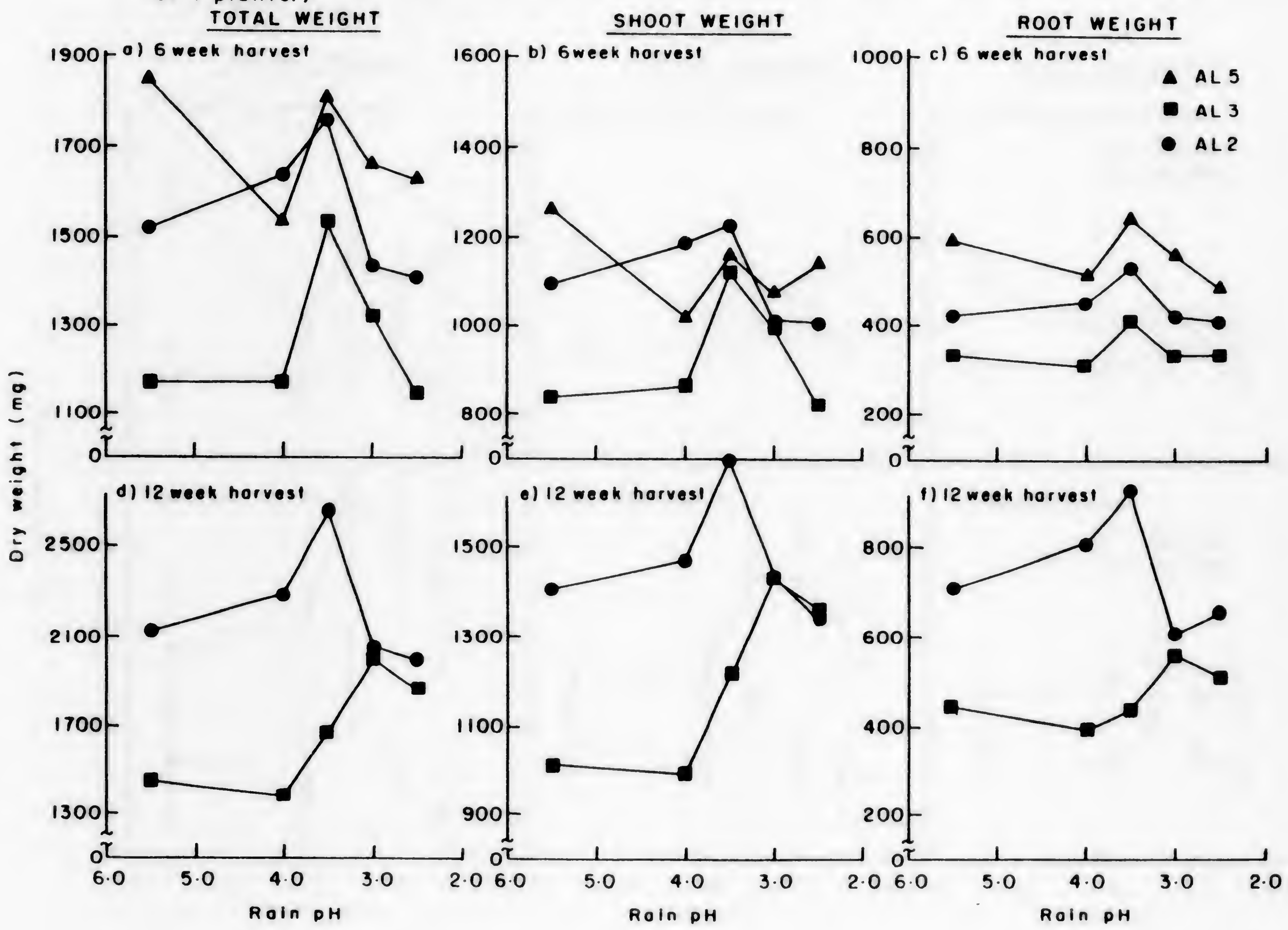
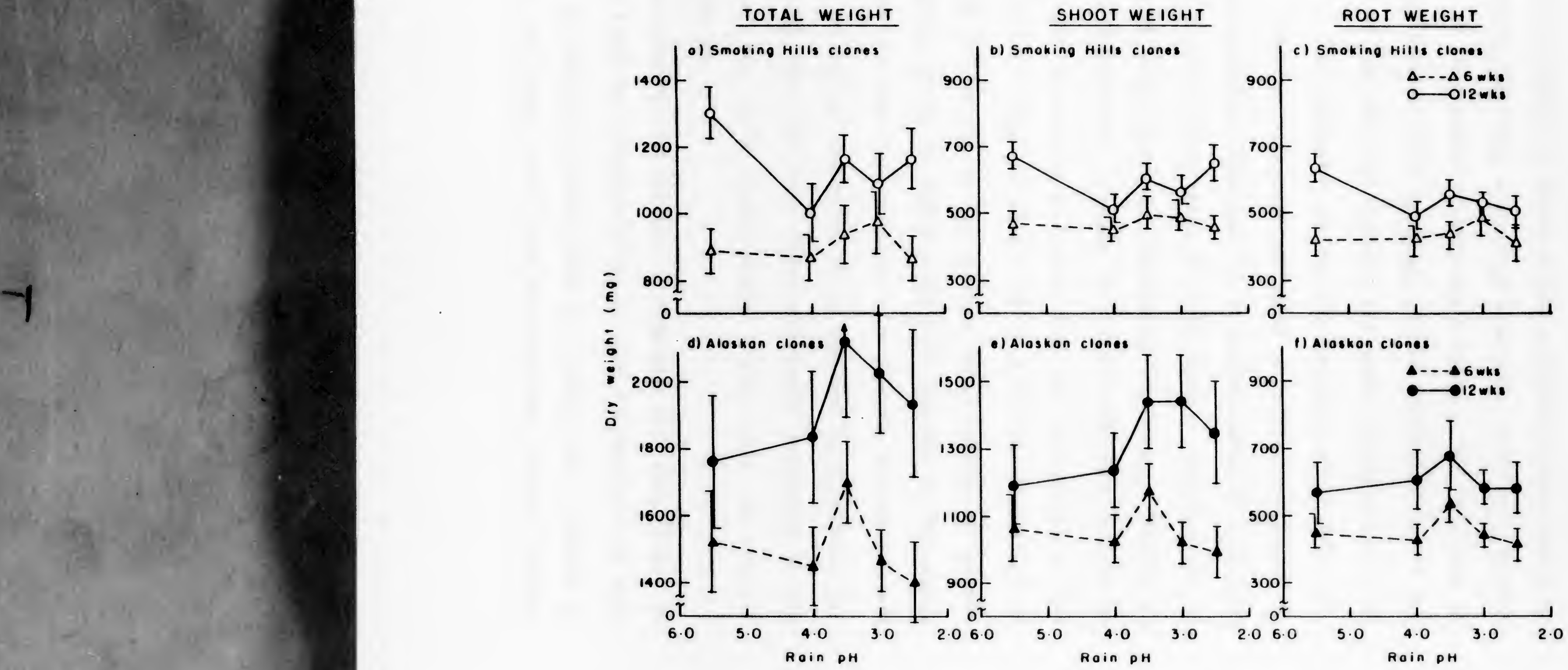


Figure 20 Effect of rainfall acidity on dry weight of Smoking Hills and Alaskan clones of Artemisia tilesii after 6 weeks and 12 weeks. (mean of 3 clones = 21 plants for each population.)



Alteration of the pH of droplets on the leaf surface

A. The buffering capacity of leaves of Artemisia tilesii, Phaseolus vulgaris and Spinacea oleracea

The ability of plants to buffer acidic droplets on the surface of leaves was investigated using a micro-pH electrode, with the capacity to measure the pH of droplets of very small volume. The responses of leaves from two representative Artemisia clones (Smoking Hills 11 and Alaska 3), and of wax bean and spinach, are shown in Figures 21 - 24 and Appendix 7. The responses are to rain droplets of pH 5.6, 3.5, 3.0, and 2.5. Solutions contained additions of cations and anions to simulate ambient rainwater (Solution "B" in methods section), and were acidified with a 2.5:1 molar ratio of H_2SO_4:H_2NO_3. Droplets had an original volume of 50 µl before any evaporation took place. Droplet pH levels showed a significant increase ($P < 0.05$) within 15 minutes of being pipetted onto the surface of leaves, when the rain droplet pH was between 5.6 and 3.0. Droplets with a pH of 5.6 were neutralized to approximately pH 7.0 for all of the species, while foliage of species exposed to droplets of pH 3.5 and 3.0 was more variable in the degree of buffering and the time for response. In general, for pH 3.5 and 3.0, there was an initial buffering of pH followed by a decline, but the pH of droplets when they were almost completely evaporated on the leaf (75 mins.) was still significantly higher than the initial rainfall solution pH.

The reasons for the variability of response with time (Figs. 22 and 23) are not known but probably relate to leaf variability, and even to differences in buffering between areas on a single leaf. Because of the possibility of leakage of K^+ from the electrode, single droplets

Figure 21

Buffering capacity of leaves after application of droplets – pH 5·6

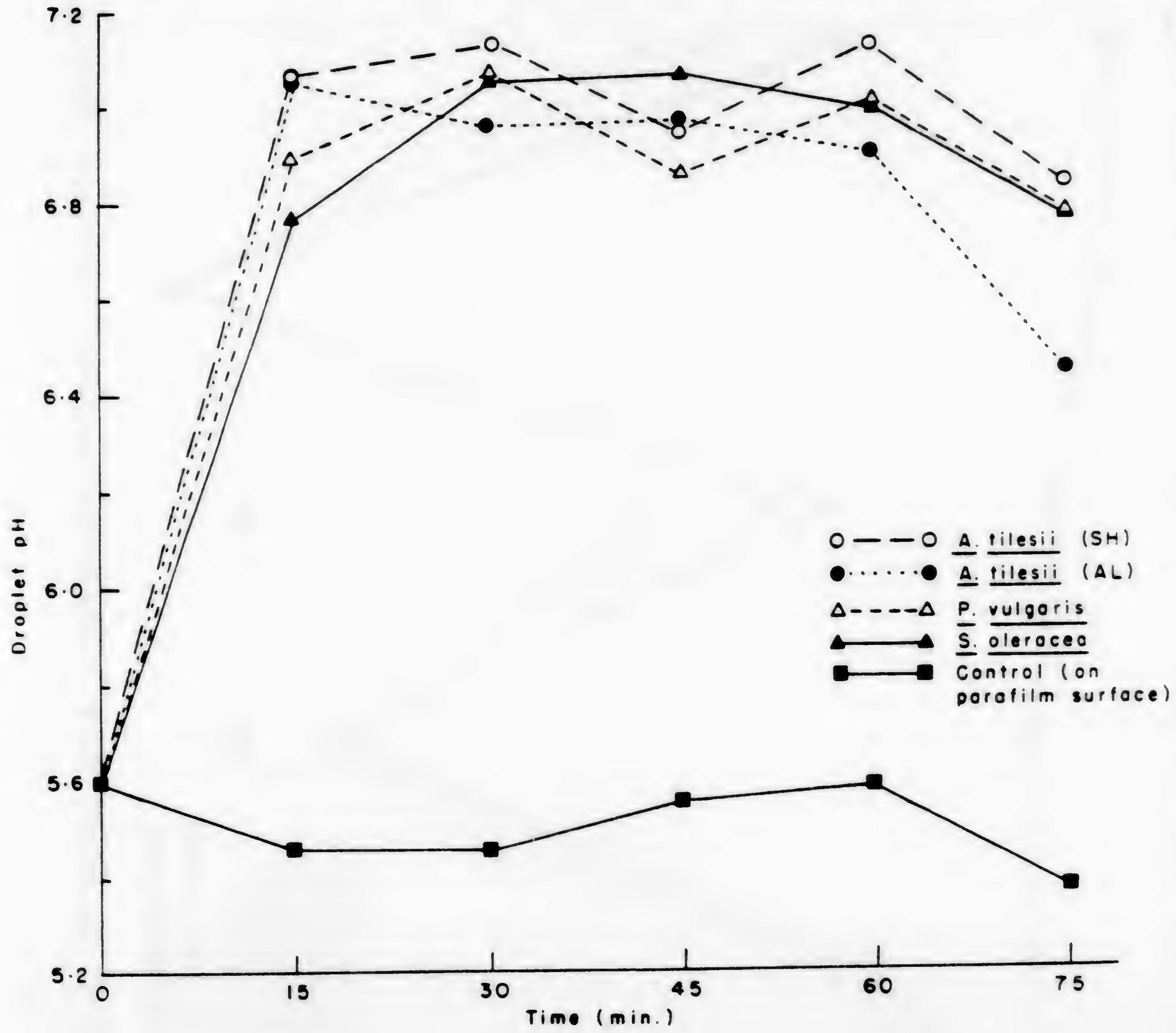


Figure 22
Buffering capacity of leaves after application of acidic droplets – pH 3·5

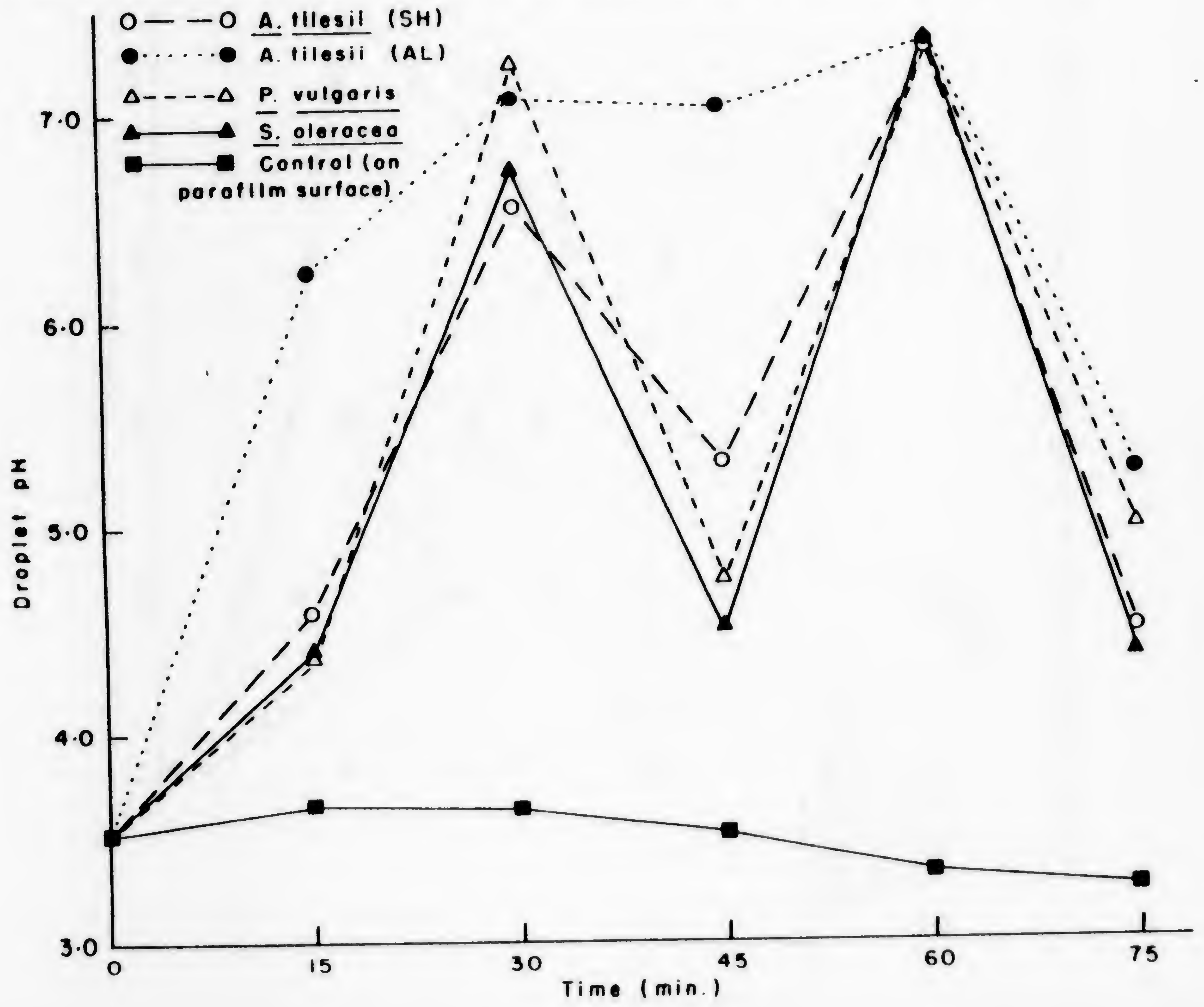


Figure 23

Buffering capacity of leaves after application of acidic droplets - pH 3·0

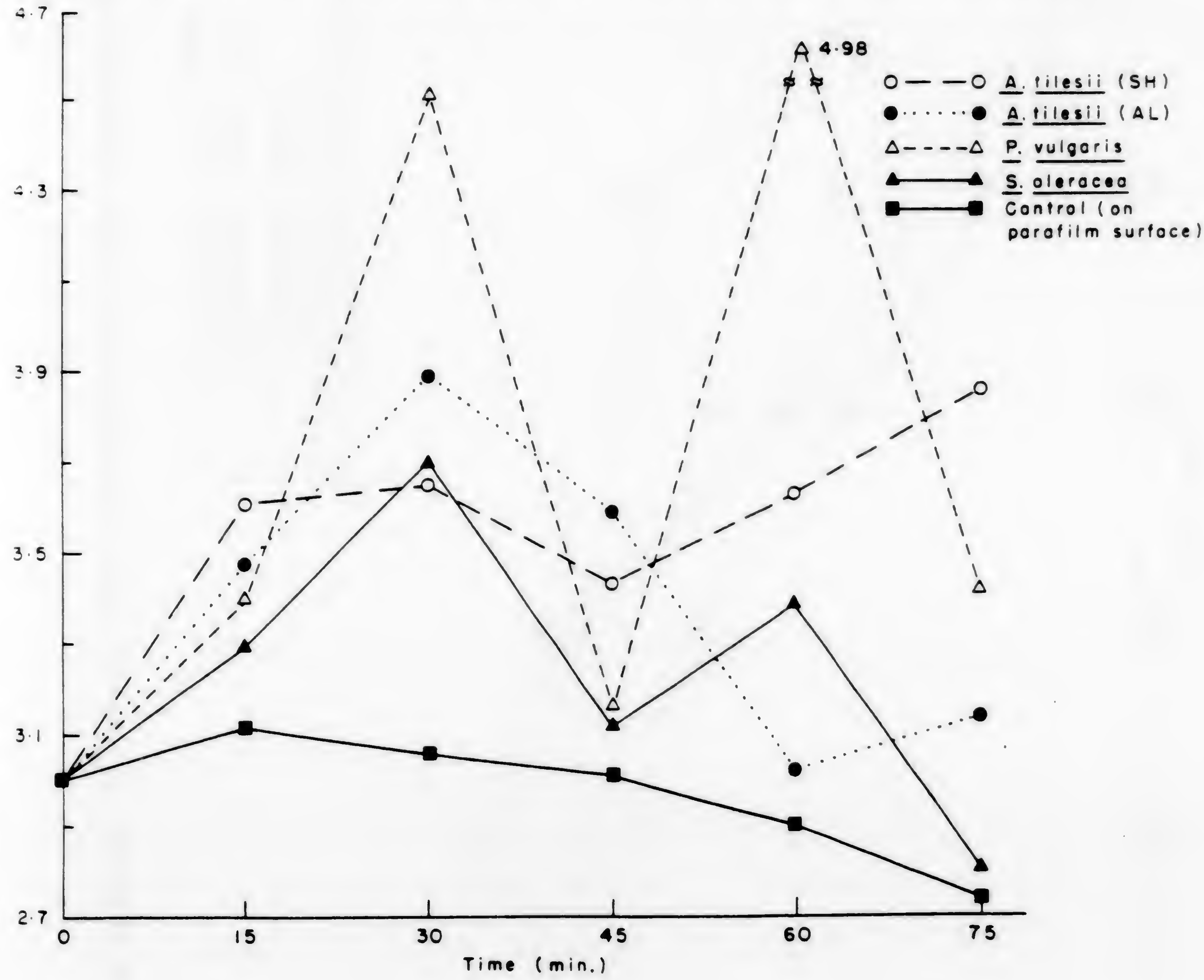
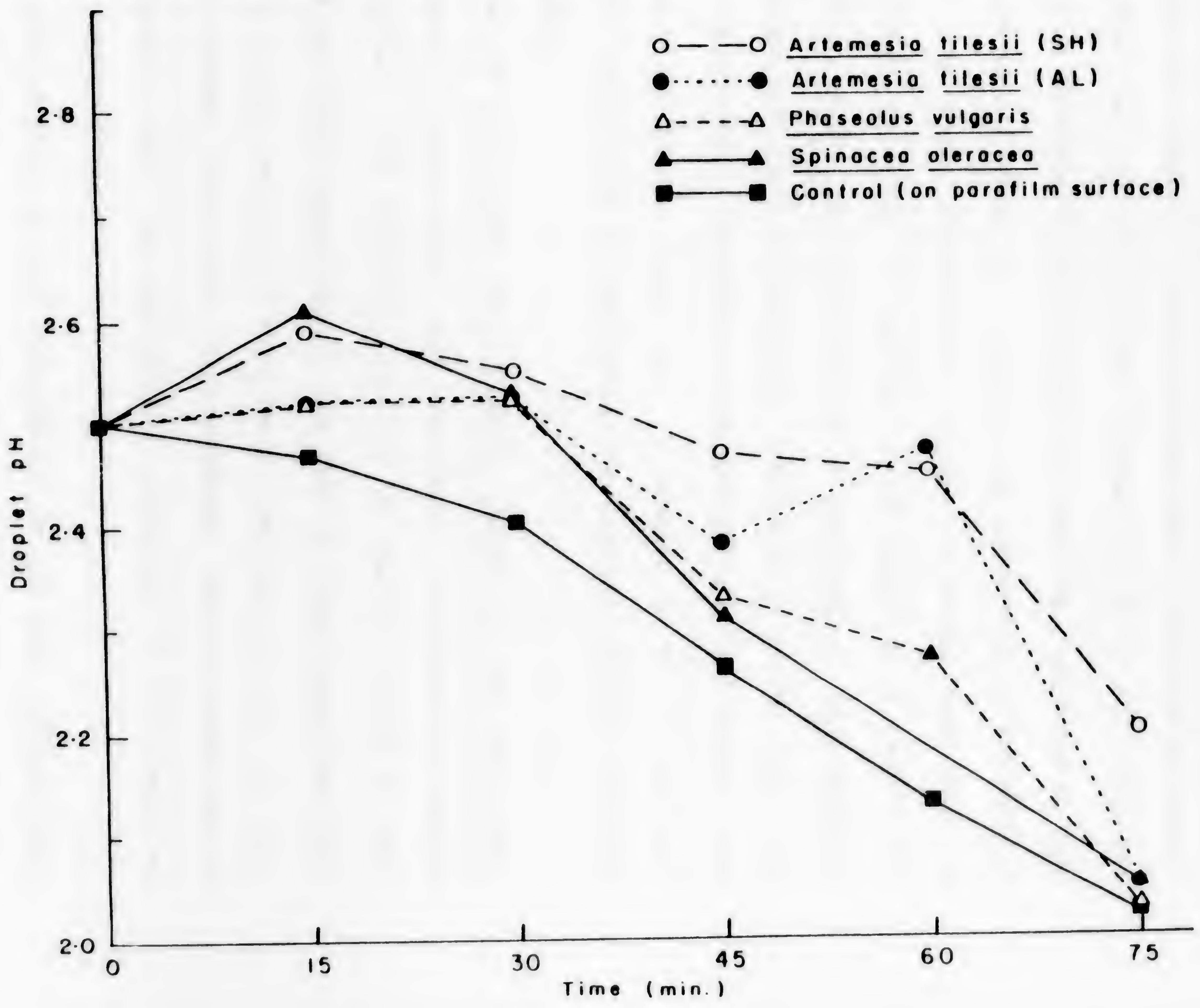


Figure 24

Buffering capacity of leaves after application of acidic droplets - pH 2·5



could be measured once only, so that droplets on a number of different leaves were measured at each time point. A small experiment was designed to study the buffering capacity of leaves of different ages and the results are given in the next section.

The response of leaves to rain droplets with a pH of 2.5 contrasts sharply with the response to rain droplets with a pH of 3.0 and above. Although there was an initial small increase in pH to 2.6, this was followed by a decrease in pH to around 2.1 over the 75 minutes it took for droplets to dry (Fig. 24). However, the droplet pH on the surface of leaves was always significantly higher ($P < 0.05$) than that of droplets placed on strips of parafilm, which served as a control for H_3O^+ increases which occur over time due to evaporation. This suggests that although the H_3O^+ concentration increased on leaves with time resulting in an overall decrease in droplet pH, the leaf surface does significantly buffer the droplet pH. A droplet of pH 2.5 delivered more acidity than could be neutralized by alkaline releases from the leaf surface.

Leaves of all plants were lightly misted in the laboratory, approximately 12 hours before each experiment, to remove any obvious dirt particles. The buffering capacity of leaves treated in this manner did not differ significantly from that of leaves which were well scrubbed two hours before the experiment removing all dust and dirt from their surface (Table 49). This indicates that the buffering of acidic droplets under our experimental conditions is not simply an effect of surface dirt.

The concentration of H_3O^+ neutralized by the leaf surface after application of rain droplets was calculated by converting the droplet

Table 49. Comparison of the droplet pH measurements made on washed[a] and unwashed[a] leaves after application of acidic droplets (pH 3.0). (Mean of droplets measured at 15, 30, 45, and 60 minutes; n=60 for unwashed leaves and n=12 for washed leaves for each species)

Species	Unwashed leaves $[H^+]$ of droplets $\bar{x}$ ± S.D.	pH $\bar{x}$	Washed leaves $[H^+]$ of droplets $\bar{x}$ ± S.D.	pH $\bar{x}$
Spinacea	0.00039 ± 0.00029	3.40	0.00064 ± 0.00025	3.19
Artemisia (Smoking Hills 11)	0.00027 ± 0.00007	3.57	0.00034 ± 0.00012	3.47
Artemisia (Alaska 3)	0.00042 ± 0.00037	3.38	0.00035 ± 0.00022	3.46
Phaseolus	0.00028 ± 0.00032	3.56	0.00009 ± 0.00010	4.03

[a] Unwashed leaves were misted with distilled water to remove any dirt particles and then allowed to dry for approximately 12 hours before buffering capacity of leaves to acidic droplets was measured. Unwashed leaves were used for all buffering capacity experiments.

Washed leaves were misted with distilled water and then each leaf was scrubbed lightly to remove any dust present and rinsed with distilled water. Buffering capacity of leaves to acidic droplets was measured as soon as the bathed leaves dried.

pH into H_3O^+ concentration and then taking the difference between the H_3O^+ concentration of droplets on the leaf surface and the initial H_3O^+ concentration of the rain droplets applied to leaves. The increase in H_3O^+ due to evaporation of droplets was determined from the H_3O^+ concentration of the droplets on parafilm strips and these were subsequently added to the value to obtain the total concentration of H_3O^+ neutralized by the leaf, or its 'buffering capacity'. The buffering capacity of leaves of Artemisia, bean and spinach is compared in Table 50 for rain droplets having a pH of 5.6, 3.5, 3.0 and 2.5. It shows a very large increase in the concentration of H_3O^+ neutralized by the leaf as the pH of the rain droplets decreased i.e. the more acidity applied, the more alkalinity released. Approximately 4000 μeq l^{-1} H_3O^+ were neutralized by Artemisia leaves after 60 minutes when the droplet pH was 2.5, compared to 1000 when the droplet pH was 3.0, 500 when the droplet pH was 3.5 and only 2 when the droplet pH was 5.6. These results show that although the pH of droplets decreased with time when rain droplets had a pH of 2.5 (Fig. 24), compared to an increase to around 7.0 when rain droplets had a pH of 5.6 (Fig. 21), the actual concentration of H_3O^+ neutralized by the leaf surface of Artemisia for droplets of pH 2.5 was 2000 times greater than for droplets of pH 5.6. The Smoking Hills clone was especially effective.

A differential ability in the capacity to buffer droplets of the three species was observed in response to acidic droplets (Table 50). Multivariate analysis of variance was used to examine the differences due to species, since it was desirable to use a model which included the pH values for all 5 sample times. Multivariate analysis of variance indicated significant differences in the neutralization of

Table 50. Concentration of H_3O^+ neutralized by the leaf surface after application of acidic droplets.

$[H_3O^+]$ neutralized by the leaf = ($\bar{x}$ droplet $[H_3O^+]$ after buffering by the leaf - $\bar{x}$ droplet $[H_3O^+]$ before placing on leaf) - ($\bar{x}$ droplet $[H_3O^+]$ on parafilm - $\bar{x}$ droplet $[H_3O^+]$ before placing on parafilm)[a]

pH of droplets applied and species	$[H_3O^+]$ μeql^{-1} neutralized by the leaf[b]				
	15 min.	30 min.	45 min.	60 min.	75 min.
pH 2.5 (3160 μeql^{-1} H_3O^+)					
Spinacea	677	1030	700	----	0
Phaseolus	117	950	820	1940	0
Artemisia (SH 11)[c]	597	1150	2100	3790	6930
Artemisia (AL 3)[d]	147	1100	1330	4050	0
pH 3.0 (1000 μeql^{-1} H_3O^+)					
Spinacea	244	674	228	1123	260
Phaseolus	354	842	310	349	1428
Artemisia (SH 11)	503	651	610	1015	1671
Artemisia (AL 3)	419	743	724	296	1085
pH 3.5 (316 μeql^{-1} H_3O^+)					
Spinacea	178	219	263	448	526
Phaseolus	176	219	277	448	558
Artemisia (SH 11)	192	219	293	448	537
Artemisia (AL 3)	216	219	293	448	557
pH 5.6 (2.5 μeql^{-1} H_3O^+)					
Spinacea	1	1	2	2	4
Phaseolus	1	1	2	2	4
Artemisia (SH 11)	1	1	2	2	4
Artemisia (AL 3)	1	1	2	2	4

[a] $[H_3O^+]$ change of droplets on parafilm was subtracted from the leaf droplet $[H_3O^+]$ to account for the concentration of $[H_3O^+]$ in droplets due to their evaporation over the 75 minute time period.

[b] Values are the mean of 15 droplets.

[c] SH 11 = Smoking Hills clone 11.

[d] AL 3 = Alaska clone 3.

Table 51. Manova tests for the effect of species on buffering of acidic droplets.

pH of droplets	Test statistic	F Values[a]	
5.6	Hotelling-lawley trace	201.4	***
	Pillai's trace	1.8	*
	Wilks' criterion	13.4	***
3.5	Hotelling-lawley trace	1497.8	***
	Pillai's trace	2.3	*
	Wilks' criterion	30.0	***
3.0	Hotelling-lawley trace	27.1	***
	Pillai's trace	5.5	***
	Wilks' criterion	14.0	***
2.5	Hotelling-lawley trace	4.2	***
	Pillai's trace	3.2	***
	Wilks' criterion	4.0	***

[a] Significance level: *P<0.05 **P<0.01 ***P<0.001

Table 52. Differences in the hydrogen ion concentration[a] of droplets on leaf surfaces of *Artemisia tilesii*, *Phaseolus vulgaris*, *Spinacea oleracea* and parafilm.

pH 2.5 rainfall $\bar{x}$ $[H_3O^+]$ μeql^{-1}	A 10,218 (Bean)	A 9,429 (Parafilm)[b]	AB 8,941 (Spinach)	AB 8,793 (*Artemisia*) AL 3[c]	B 6,205 (*Artemisia*) SH 11[d]
pH 3.0 rainfall $\bar{x}$ $[H_3O^+]$ μeql^{-1}	A 1,812 (Parafilm)	A 1,559 (Spinach)	B 722 (*Artemisia*) AL 3	BC 385 (Bean)	C 140 (*Artemisia*) SH 11
pH 3.5 rainfall $\bar{x}$ $[H_3O^+]$ μeql^{-1}	A 568 (Parafilm)	B 42 (Spinach)	B 31 (*Artemisia*) SH 11	B 10 (Bean)	B 5 (*Artemisia*) AL 3
pH 5.6 rainfall $\bar{x}$ $[H_3O^+]$ μeql^{-1}	A 4 (Parafilm)	B 0.3 (*Artemisia*) AL 3	B 0.2 (Spinach)	B 0.2 (Bean)	B 0.1 (*Artemisia*) SH 11

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[a] Values are the mean H_3O^+ concentration of 15 droplets, after 75 minutes on the leaf surface.

[b] Strips of parafilm were used as a control - see text for explanation.

[c] AL 3 = Alaska clone 3.

[d] SH 11 = Smoking Hills clone 11.

NOTE: Means having the same letter do not differ significantly ($\alpha = 0.05$, Duncan's Multiple Range Test).

H_3O^+ due to species, using 3 test statistics all related to the characteristic roots and vectors of the matrix $ERROR^{-1}$ HYPOTHESIS (Table 51). To examine these species differences further, Duncan's Multiple Range tests were applied to the mean droplet $[H_3O^+]$ values at 75 minutes, the point at which the droplets were almost dry (Table 52). Duncan's Multiple Range tests indicated that for acid rain droplets with a pH of 3.0 and 2.5, there was a significant difference between the species in the ability to neutralize H_3O^+. Acidic rain droplets on the leaf surface of the Artemisia clones were neutralized significantly more than acidic droplets on leaves of spinach or bean. Spinach foliage had the poorest ability to buffer acidic droplets, and when the droplets were almost dry, the $[H_3O^+]$ was as high as droplets on the control parafilm strips. For droplets having a pH of 5.6 or 3.5, Duncan's Multiple Range tests indicated that there were no significant differences between the species in their ability to neutralize H_3O^+. However, the leaves of all of the species had a significantly higher ability to buffer the $[H_3O^+]$ than did the control parafilm strips.

These results indicate that foliage of Artemisia has a greater ability to neutralize acidic droplets than either bean or spinach. In studies of foliar injury to these same species (page 74), the ability to buffer acidic droplets correlates with the amount of injury observed on the foliage. The greater the buffering capacity of leaves, the less foliar injury from acid droplet exposure.

B. The effect of leaf age on buffering capacity

As the age of spinach leaves increased, a marked increase in neutralization of H_3O^+ in droplets on the foliage was observed

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(Fig. 25, Appendix 8). Young spinach plants (5 weeks), having five leaves, were used in this experiment. Neutralization of acidic droplets was observed to be highest on the two oldest spinach leaves. Age of leaves was probably involved in the fluctuation of [H_3O^+] in droplets over time for bean and spinach, observed in earlier experiments (Figs. 22 and 23). The highest pH values for these species occurred at the 30 and 60 minute time intervals and these were the values of droplet pH sampled on the two oldest leaves.

Figure 25 Effect of leaf age on the ability to buffer acid droplets (pH 3·0).
(Leaves are numbered in order of increasing age.)

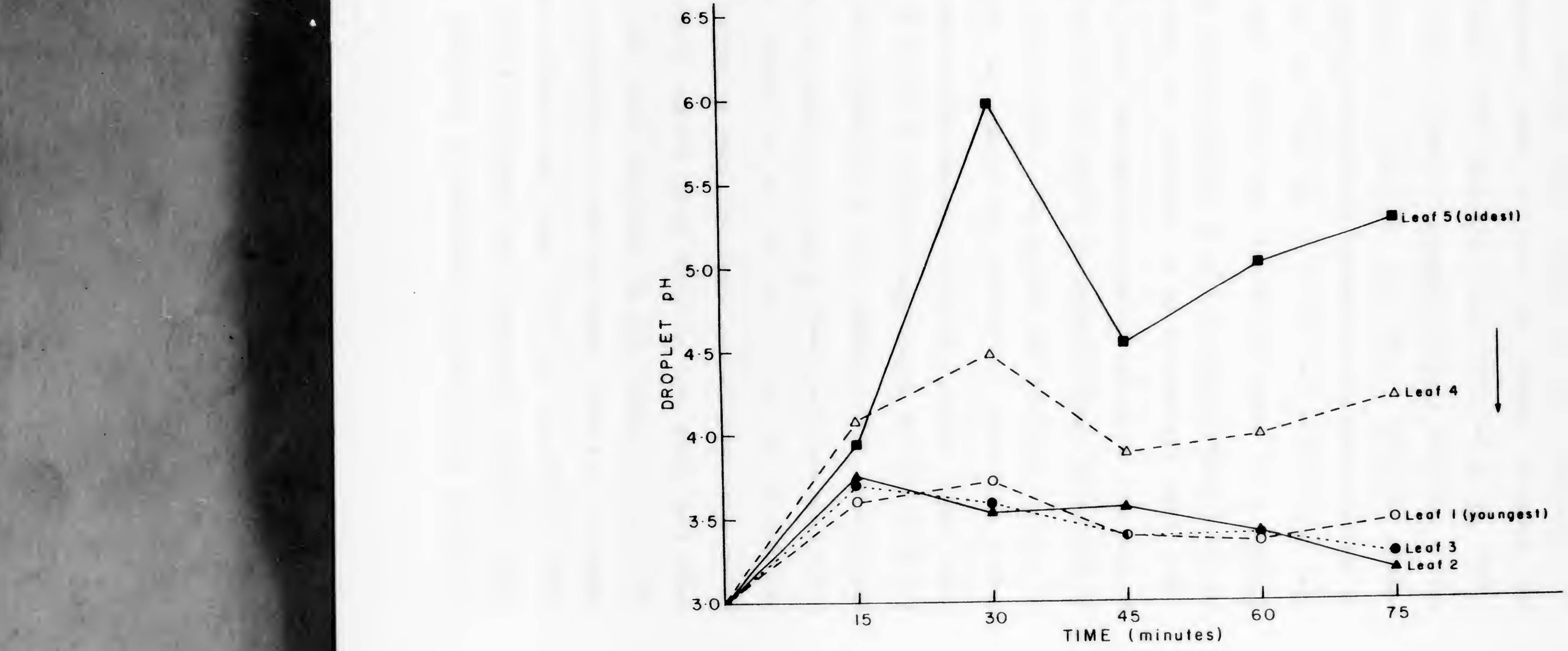
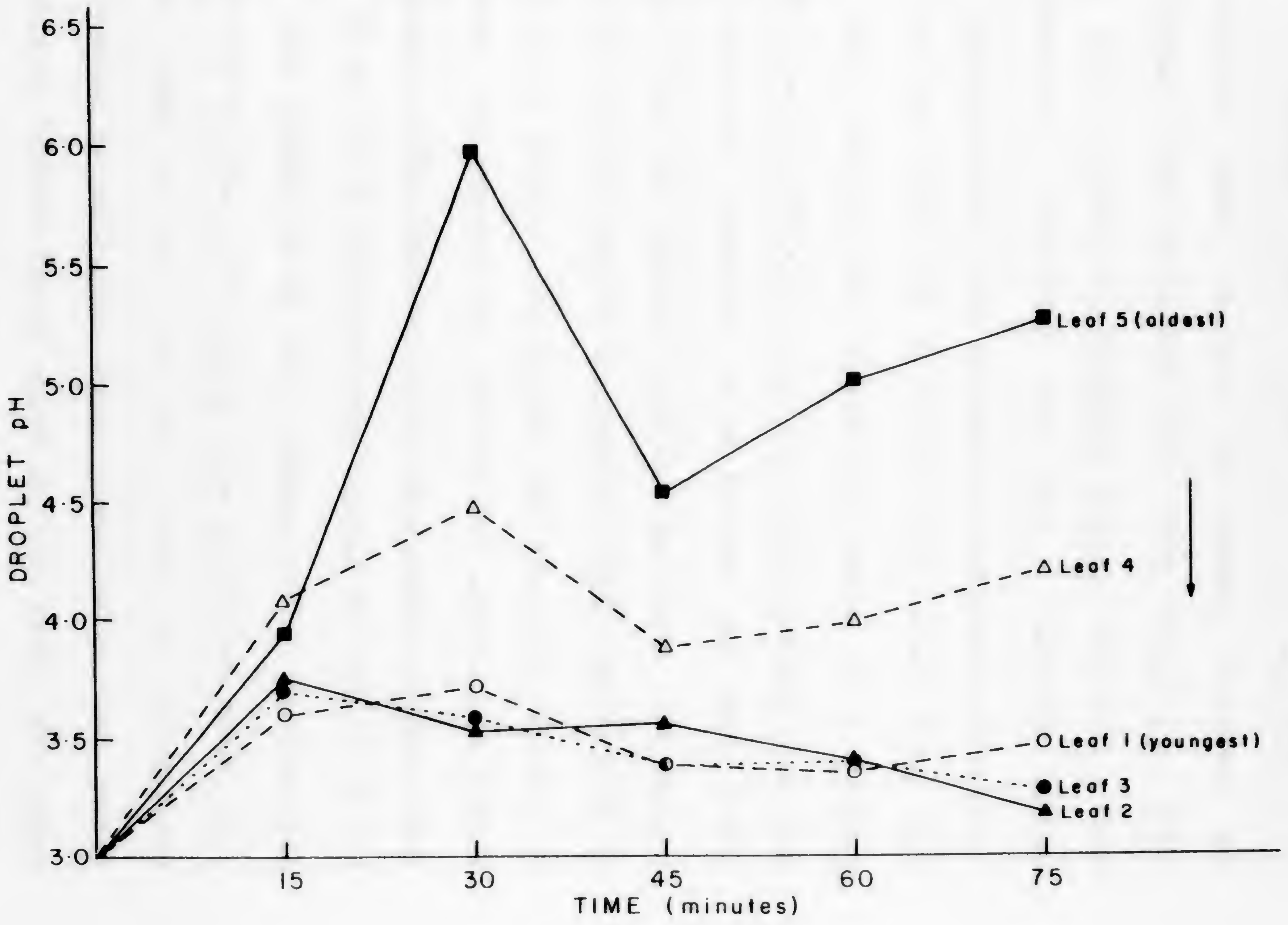


Figure 25 Effect of leaf age on the ability to buffer acid droplets (pH 3·0).
(Leaves are numbered in order of increasing age.)



DISCUSSION

Contrary to numerous other reports of the sensitivity of broadleaved herbaceous plants to acid rain damage, (Jacobson and Leuken, 1977; Mohamed, 1978; Evans and Curry, 1979; Lee et al, 1981; Johnston et al, 1982), foliage of Artemisia tilesii exhibited a high degree of resistance to simulated acid rain. Necrotic lesions developed on A. tilesii foliage from exposure to simulated acid rain at pH 2.5 after one rain event and at pH 3.0 only after three rain events. While small lesions were observed on leaves of A. tilesii after exposure to several rainfalls at pH 3.0, the percentage leaf area injured after 12 weeks (24 rainfalls) at pH 3.0 was very small (<2%). No visible foliar injury was observed at pH 3.5 and above. The data of Lee et al (1981) for 22 crop species all showed a higher sensitivity of these species to foliar injury. The percent leaf area with lesions, at the time of harvest, was greater than 10% for four species and greater than 5% for 15 species after exposure to pH 3.0 rainfall in the field. In their study, 28 out of 35 cultivars were injured by rain at pH 3.5 and 5 cultivars were injured at pH 4.0. Mohamed (1978), working with many of the same crop species as Lee et al under greenhouse conditions, observed foliar injury at pH 4.0 to 11 out of 13 species.

It is interesting to note that where investigators have worked with the same species, visible injury occurs at less acidic pH's under greenhouse than under field conditions, in some instances (Table 53). This increased susceptibility to acid rain injury indoors is consistent with data reviewed in Martin and Juniper (1970) and from Shriner (1981) suggesting that cuticle development under temperature, humidity, light, and air movement conditions in greenhouses is significantly altered. It

Table 53. Summary of the results from recent experiments on effects of simulated acidic rain on vegetation.

Plant group and species	Experimental conditions	pH of rainfall at which foliar injury was observed	Investigator
CONIFERS			
Pinus sylvestris (Scots pine)	greenhouse	3.0 (short needle & tip injury)	Hindawi & Ratsch (1974)
Pinus strobus (eastern white pine) *P. taeda* (loblolly pine)	greenhouse or field	3.2 (after 2 months) 1.9 > 2.7 (after 40 minutes)	Shriner *et al* (1975)
Pinus sylvestris *P. strobus*	field	none at pH 3.0	Wood and Pennypacker (1975)
Pinus sylvestris *P. strobus* *P. ponderosa* (Ponderosa pine)	greenhouse	3.5 (short needle)	Gordon and Tourangeau (1975)
Pinus sylvestris	field	2.5	Abrahamsen *et al* (1976)
Pinus strobus	greenhouse	2.3 (none at 3.0)	Wood and Bormann (1977)
Pinus strobus *P. sylvestris*	greenhouse	not given- "conifers were more tolerant than herbaceous species used in this study"	Jacobson and Leuken(1977)
WOODY DICOTS			
Betula alleganiensis (yellow birch)	greenhouse	3.0	Wood and Bormann (1974)
Acer saccharum (sugar maple)	greenhouse	3.0	Wood and Bormann (1975)

Continued

Table 53. (Continued).

Plant group and species	Experimental conditions	pH of rainfall at which foliar injury was observed	Investigator
WOODY DICOTS (con't)			
Betula pubescens (birch)	field	2.5	Abrahamsen *et al* (1976)
Quercus phellos (willow oak)	greenhouse	3.2	Lang *et al* (1978)
Populus sp. (poplar)	greenhouse	3.4	Evans *et al* (1978)
Quercus palustris (pin oak)	greenhouse	2.5 (< 1% leaf injury)	Evans and Curry (1979)
HERBACEOUS			
Phaseolus vulgaris (bean)	greenhouse	2.5	Ferenbaugh (1974)
Phaseolus vulgaris (kidney bean) *Glycine max* (soybean)	greenhouse or field	3.2	Shriner *et al* (1975)
Phaseolus vulgaris (pinto bean)	greenhouse	3.0	Wood and Bormann(1975)
Helianthus annuus (sunflower)	greenhouse	3.4 (9 hr. spray)	Jacobson and Leuken (1977)
Phaseolus vulgaris (bean)		"	
Spinacea oleracea (spinach)		"	
Phaseolus vulgaris (bush bean)	greenhouse	3.0	Hindawi *et al* (1977)
Glycine max	greenhouse	3.0 (2 cm)	Irving (1978)
Glycine max	field	none at 3.1	Irving and Miller (1978)

Continued

Table 53. (Continued).

Plant group and species	Experimental conditions	pH of rainfall at which foliar injury was observed	Investigator
HERBACEOUS (con't)			
13 crop species	greenhouse	all were injured at 3.0, 11 out of 13 were injured at 4.0	Mohamed (1978)
Tradescantia sp. (spiderwort)	greenhouse	2.5	Evans and Curry (1979)
Pteridium aquilinum (bracken fern)		3.4	
Glycine max		3.4	
35 crop species	field	31 out of 35 injured at pH 3.0, 28 out of 35 injured at pH 3.5, 5 injured at pH 4.0 (monocot species were most tolerant of all of the crops)	Lee *et al* (1981)
Senecio vulgaris (common groundsel)	greenhouse	2.5	Hodgkin & Briggs (1981)
Phaseolus vulgaris (bush bean)	greenhouse	3.2 - 4.0	Johnston *et al* (1982)
Artemisia tilesii	greenhouse	3.0 (< 1% leaf injury)	Adams (present study)

could also be due to increased neutralization of acidic droplets by alkaline particulates and soil particles deposited on the surface of leaves under outdoor conditions.

The sensitivity of *Phaseolus vulgaris* "7 pencil pod wax" (wax bean) and *Spinacea oleracea* "Longstanding bloomsdale" (spinach), which have been studied previously by other investigators, was also determined in this study to: (1) see how their sensitivities under the experimental conditions used for *Artemisia tilesii* compared with their sensitivities reported in the literature, and (2) to compare the sensitivities of other herbaceous species with that of *Artemisia* foliage. Similar results to previous studies (Ferenbaugh, 1974; Jacobson and Leuken, 1977; Lee *et al*, 1981) were obtained, in which foliage of spinach was very sensitive to acidified rain solutions and bean exhibited less injury. Leaves of *Spinacea oleracea* developed necrotic spots, 1 - 4 mm, in diameter on 20 - 40% of the leaf area after as little as 4 applications (total of 4 cm) at pH 2.5. Both species were more sensitive to acid rain than were clones of *Artemisia tilesii* from either Alaska or the Smoking Hills.

Evans (1981) has reviewed the literature on foliar injury and observed that: herbaceous species are the most sensitive of all plant groups, followed by woody dicotyledons, monocotyledons and conifers, in that order. Threshold rain pH values reported in the literature for visible foliar injury are summarized in Table 53. These data are generally consistent with the view of Evans. Visible injury to foliage is induced between pH 3.0 - 4.0 for herbaceous species, pH 3.0 - 3.4 for deciduous tree species and pH 2.3 - 3.0 for coniferous tree species. The ground flora, excluding bryophytes and lichens, in coniferous forest ecosystems, as well as the conifers themselves, may also have a high degree of tolerance to acid rain. The reasons for this are that these species are very likely adapted to podsolic soils with a low pH (Hutchinson, 1981) and throughfall beneath coniferous canopies is normally acidic due to leaching of tannic acids, fulvic acid etc. from the needles. Research is necessary to determine the response of ground flora species from coniferous forest ecosystems to simulated acid rain. The present Burt Lake programme of T. Hutchinson and that of the Canadian Forest Service group under K. Percy should answer this.

The difficulty in evaluating the sensitivity of herbaceous plant species to acid precipitation is the overwhelming bias in using crop species in simulated acid rain experiments. Only three native herbaceous species had been investigated prior to these studies with Artemisia tilesii. Two of these species, Senecio vulgaris (common groundsel) and Tradescantia (spiderwort), were quite insensitive to acidified rain solutions. Injury occurred to both of these species only at pH 2.5 and below (Hodgkin and Briggs, 1981; Evans and Curry, 1979). Experiments with Artemisia tilesii also showed a high tolerance of this species to simulated acid rain. Even though only a very small number of native species have been looked at, these data suggest that native herbaceous species may be more resistant to acid precipitation than crop plants. Thus, there may be a lack of evidence to support the contention that as a group, herbaceous species are highly sensitive to acid precipitation. More native species should be tested to verify whether they are more tolerant than crop species. In addition a comparison of the sensitivity of annuals and perennials merits attention to determine if features, such as cuticle thickness, coniferous forest ecosystems, as well as the conifers themselves, may also have a high degree of tolerance to acid rain. The reasons for this are that these species are very likely adapted to podsolic soils with a low pH (Hutchinson, 1981) and throughfall beneath coniferous canopies is normally acidic due to leaching of tannic acids, fulvic acid etc. from the needles. Research is necessary to determine the response of ground flora species from coniferous forest ecosystems to simulated acid rain. The present Burt Lake programme of T. Hutchinson and that of the Canadian Forest Service group under K. Percy should answer this.

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Experiments were performed to compare the sensitivity of *Artemisia tilesii* from a "control" (unpolluted) population with *A. tilesii* from the Smoking Hills. The results of the comparison failed to show that clones from the Smoking Hills population, which came from an area with very high levels of SO_2 and acid aerosol, were more tolerant of the simulated acid rain treatments than clones from the Alaskan ("control") population. In general, the percent leaf area with lesions and the percentage of leaves injured were variable among all nine clones for rainfall at pH 2.5, but the sensitivity of individual clones from the two populations overlapped. Both populations showed a high tolerance. Though the number of clones sampled was very small, it is tempting to believe that the species as a whole may be pre-adapted to acid rain conditions, and that its tolerance relates to characteristics essential for the normal maintenance of the species in its environments.

Variation in the percentage of leaves which were injured for the six Smoking Hills clones related quite closely to their morphological characteristics. Clones which resembled each other showed very similar percentages of injured leaves. The clones which showed the greatest sensitivity to foliar lesions (clones 3 and 9) had a trailing growth habit, with leaves produced at further intervals from each other along the stem than any of the other clones. Because of this, rainfall had a greater probability of wetting all or most of the leaves on a plant. The leaf orientation of these two clones, which differed from that of other clones, was also a contributing factor to their high sensitivity. Their leaves clasped the stem at the leaf base and were oriented upwards, favouring the retention and pooling of water, particularly in nodal areas and along veins. Leaf abscission was observed in these clones as a result of damage to leaf tissues at the leaf base. The two clones which were least injured (clones 11 and 12) had leaves which curved downwards at their ends, favouring the droplets rolling off the leaves. This contrasted with the vertically oriented leaves of clones 3 and 9, which favoured the pooling of solutions, and the horizontally positioned leaves of clones 5 and 8 which retained droplets for a longer time.

These results confirm the hypothesis of Jacobson (1980) that morphological characteristics of leaves are important in determining sensitivity of plants to acid precipitation. In clones of Artemisia tilesii, plant stature, leaf size and shape, the amount of leaf dissection and leaf orientation were identified as the critical features responsible for the percentage of leaves which were injured.

Although morphological characteristics were closely related to the number of leaves which had lesions from acid exposure, no such relationship occurred with the amount of surface area injured on leaves. The observed differences in the percent of leaf area with lesions among clones of Artemisia tilesii could be related to features that determine leaf wettability, such as cuticle structure, or to histological responses that prevent the spread of injury by reducing intercellular space, or to the buffering capacity of leaves. Knowledge of which characteristics of a plant impart resistance (or sensitivity) is extremely important since it could then be used to predict those tree, understory, and crop species which are most at risk from acid precipitation. Breeding of crops for acid rain resistance will become a more practical and less empirical proposition when the factors that determine foliar response are better understood. Further research in this area is needed.

Measurements of plant productivity (number of new leaves produced and dry weight) did not show a significant difference in the response of clones from the Smoking Hills and Alaskan populations to simulated acid rain. While the foliar injury and plant productivity results suggest that the characterisitics which adapt *A. tilesii* to acidic rain are inherent in this species, further studies testing a greater number of clones, and clones from other populations, are necessary before categorical statements can be made that selection and ecotypic differentiation has or has not occurred in response to the acidic environment at the Smoking Hills. Evidence of ecotypic differentiation in response to acid rain has recently been reported in populations of *Senecio vulgaris* occurring in heavily polluted areas in Great Britain (Hodgkin and Briggs, 1981). Earlier investigations have established that SO_2 resistance occurs in some plants from heavily polluted areas (Bell and Mudd, 1976; Horsman *et al*, 1978).

It is interesting to point out that genetic differentiation has occurred in *Artemisia tilesii* at the Smoking Hills in response to abiotic factors resulting in plants which are very dwarfed and have much smaller leaves in comparison with *A. tilesii* plants from other locations. This dwarfness has been maintained in the Botany greenhouse at the University of Toronto for two years. These adaptations may be the result of selection caused by the fumigations of SO_2 from the burning shale cliffs or they may be adaptations to a coastal environment, i.e. to factors such as exposure and high wind speeds.

Plants from the Smoking Hills also differed in leaf anatomy from the Alaskan plants. The leaves of Smoking Hills plants were thicker, had one more layer of palisade parenchyma, a greater amount of spongy parenchyma, generally larger cells, and more intercellular space. These anatomical changes are typical of those ascribed to plants growing in saline environments (Waisel, 1972; Poljakoff-Mayber, 1975). Unfortunately, there is very little experimental evidence to show whether these features of halophytes are genetically fixed or are induced under saline environments. There have also been no studies of the morphological and anatomical changes which are associated with SO_2 resistance.

The presence of stomata on the adaxial surface of leaves of Smoking Hills plants (which were absent on Alaskan plants) is not easily explained on the basis of either salinity or SO_2 resistance, since it does not appear to be adaptive to either situation. Indeed, plants of coastal ecotypes generally have fewer stomata per unit leaf area (Waisel, 1972).

The data collected from the dose-response experiments showed that a number of characteristics of the rain event itself, such as rainfall intensity, rainfall duration, frequency of rain events, and droplet size, may influence foliar response to acidic rain. The trends of (1) increasing duration of rainfall and (2) decreasing time period between rain events, showed that both of these factors increased the production of foliar lesions on Artemisia tilesii. The evaluation of the importance of rainfall duration and rainfall frequency on plant response is made more difficult by the fact that the magnitude of the effect of these factors was altered by rainfall pH. At acute hydrogen ion concentrations (pH 2.0) increasing the duration of the rainfall event significantly increased the amount of leaf area damaged, while increasing the frequency of rainfall exposures from every other day to every day had no effect on the amount of foliar injury. In contrast, at pH 3.0 increasing the duration of the rainfall event had no effect on the amount of injury observed. Injury increased when plants were sprayed daily with pH 3.0 rather than every other day.

One possible explanation for the difference in effect at pH 3.0 and 2.0 is that when rainfall is moderately acidic (pH 3.0) plants may be able to cope with rainfalls of longer duration by neutralizing the droplets, either by transport of cations within the leaf onto the leaf surface, or from alkaline deposits of dust and salts on the leaf surface. For rainfalls of greater acidity this neutralizing capacity would be rapidly depleted so that greater amounts of injury would be expected from rainfalls of longer duration. Jacobson and Leuken (1977) reported that foliar injury to three crop species was induced at less acidic rain pH's when the treatment duration was increased. Foliar injury occurred at pH 2.6 or below for a treatment duration of 1 minute, pH 3.0 or below for a total duration of 1.5 hours, pH 3.2 for a total duration of 3 hours, and pH 3.4 or below for a total duration of 9 hours. The length of the rain event is probably of great importance in determining foliar response for natural rain events of low pH, which may last several hours or a few minutes. Injury will increase when leaves no longer have the capacity to buffer acidic droplets on the leaf surface.

Frequent rain events may also increase the possibility of injury at rain pH's where injury would not occur when longer periods of time elapse between rain events. This "recovery period" may serve to restore levels of cations in leaves which become depleted during an acidic rain event. The length of time needed for 'recovery' would be dependent on the concentration of cations that was lost from the leaf tissue. Alternatively, it may allow recovery of plants from injury through processes of repair and compensatory growth (Jacobson and Triano, unpublished).

Microscopic examination of leaves of *Artemisia tilesii* showed that cellular injury of foliage from simulated acid rain occurs in the absence of visible foliar injury. Scanning electron micrographs and leaf clearings showed the presence of small lesions, consisting of from one to a few collapsed epidermal cells, from exposure of plants to rainfall acidities (pH 3.5) above the threshold values necessary to produce visible lesions on leaves. To my knowledge there have been no reports of visible plant injury in the field situation [except one case of volcanic origin (Kratky *et al*, 1976)], even though rain events as low as pH 3.0 are regularly recorded. Anatomical investigations could prove to be a more sensitive indicator of acid rain injury to vegetation from exposure to natural acid rain or mist, though, of course, they may not relate to economic injury.

SEM examination revealed significant differences in stomatal aperture size between *Artemisia tilesii* plants treated with acidified rain solutions (pH 2.5) and those treated with rain of pH 5.6 (control). In injured areas, on the adaxial leaf surface, stomata were open in contrast with the closed stomates in the adjacent uninjured tissue and on control leaves (Plates 11 - 14). Drying out of the epidermal cells in necrotic tissues probably results in shrinkage of guard cells and permanent opening of the stomata. The number of open stomata was quite large in some instances where extensive injury had occurred after 8 rainfalls of pH 2.5. These results suggest that the proposal made several years ago by Tamm and Cowling (1977), that acidic precipitation might affect the rate of gas exchange, is correct. Evans *et al* (1981a) reported decreased diffusion resistance in foliage of *Phaseolus vulgaris* exposed to simulated rain of pH 2.7 to 3.4 (no visible injury at pH 3.4) compared with foliage exposed to rain of pH 5.7. The reasons for the reduction in adaxial leaf resistance, which were unknown to the author, may be related to the opening of the stomata on adaxial leaf surfaces. The open passage to internal leaf tissues, caused by damage to the guard cells, would undoubtedly interfere with gas-exchange reactions. The effect of acidic precipitation on the normal functioning of guard cells also suggests that acid-injured foliage may be more susceptible to infection by epiphytic plant pathogens, to wilting or drought stress, as well as being more sensitive to gaseous air pollutants. The SEM observations could explain the results of two previous investigations which reported an interaction of acidic precipitation with ozone in causing effects on vegetation (Shriner,1978b; Jacobson *et al*, 1980). Entry of ozone into the leaf would be facilitated by the damaged and open stomata. Acidic components in subsequent rainfall may also penetrate through resultant openings.

It has been postulated by Evans that injury from acidic rain occurs preferentially at the site of certain leaf surface features, such as trichomes, guard and subsidiary cells of stomata, and along veins (Evans et al, 1977a, b; 1978; Evans and Curry, 1979). In a variety of plant species studied by Evans, about 95% of all lesions occurred at these sites. Measurements of the percentage of lesions occurring over vascular tissue on leaves of Artemisia tilesii support this view. A significantly higher percentage of lesions occurred over vascular tissue than would be expected based on the ratio of the area of the leaf surface occupied by vascular and non-vascular tissue. In these calculations (refer to Appendix 5) only lesions <100 µm (approx. 2 cells in size) were considered, in order to be certain that the lesions had actually developed initially at the site of a vein. Higher percentages of lesions occurred over vascular tissue if larger sized lesions (250 - 1000 µm) were also included in the measurements, indicating that some lesions increase in size to cover vascular tissue. The reason for lesion localization over veins is thought to be because these form natural depressions in the leaf surface allowing rainwater to pool, or alternatively that the cuticle is thinner over veins. More work is needed to determine the preferential sites of lesion development on leaves of a wide variety of species. Care should be taken to use lesions in the smallest size range (<100 µm) since larger lesions which include a stomate, vascular tissue or a trichome may not necessarily have originated at these structures. An understanding of the sites of lesion initiation can help predict the characteristics of plant species which make them susceptible to acid rain injury.

Lesion development in Artemisia tilesii exhibited two diverse responses after exposure to acidic rain. In most lesions, injury began on the adaxial epidermis and affected internal tissues successively toward the abaxial epidermis. Exposure to several acid rainfalls or to a single rain event of high acidity resulted in complete collapse of all tissue layers. Lesion development in Artemisia tilesii was very similar to the development of lesions in leaves of Phaseolus vulgaris, Helianthus annuus, Pteridium aquilinum and Populus sp. (Evans et al, 1977a, 1978; Evans and Curry 1979; Evans, 1980). However, in some leaves internal injury (hypertrophy and hyperplasia) was observed without collapse of the overlying epidermal cells. This pathway of lesion development was observed in less than 10% of the sectioned leaves where damage had occurred. The mechanism for this response is not known. Perhaps the permeability of epidermal cells was altered by acidic solutions which allowed acidic rainwater to penetrate internal tissues. The high affinity of the epidermal cells over the injured area for Paragon Blue stain, which entered these cells readily, is supportive of this hypothesis. The staining reactions of these cell walls are suggestive of oxidized phenolic material rather than the normal cellulose staining response of healthy tissues. This pattern of lesion development is of particular interest because it results in abnormal cellular events in mesophyll cells and most probably macroscopic injury symptoms do not develop.

If one compares the amount of leaf area damaged at the cellular level with the amount of macroscopic injury observed, the levels of cellular injury are higher (by up to an order of magnitude), in all cases (Tables 23 - 25). These results indicate that visual estimates of leaf surface injury may significantly underestimate the damage to leaves from acid rain. Cellular injury from acidic rain was both more extensive than macroscopic injury and occurred when rainfall was less acidic.

An increase in the amount of leaf area injured as a result of repeated exposure to acidic rain has been recognized (Evans *et al*, 1978; Evans and Curry, 1979). Studies of the pattern of development of lesions in *Artemisia tilesii* show this increase in area injured to be a function of both an increase in the size of lesions already present on the leaf and the development of new lesions. Depressions in the leaf surface which form as a result of tissue collapse probably serve as an area for collection of subsequent rain (Evans, 1980).

One of the objectives of the histological work was to see whether hypertrophy and/or hyperplasia occurred in mesophyll cells as a result of acid rain exposure. Very few histological studies of injury from acid rain have been carried out (Evans *et al*, 1977a; Evans *et al*, 1978; Evans and Curry, 1979; Evans, 1980). From preliminary work Evans has suggested that, in general, plant species which exhibit extensive hyperplastic and hypertrophic responses are more tolerant than species that do not have these responses. Foliage of *Artemisia tilesii* is quite insensitive to simulated acid rain. The occurrence of both hypertrophy and hyperplasia in mesophyll cells of *A. tilesii* leaves is consistent with the view that hyperplastic and hypertrophic responses of foliage may alleviate extensive foliar injury. In leaves of *Populus* and *Tradescantia* these rapid cell divisions and cell enlargements produced a gall which elevated the epidermis, thus reducing subsequent injury by preventing pooling of droplets in these areas (Evans *et al*, 1978; Evans and Curry, 1979). Such "gall formations" formed only occasionally in foliage of *A. tilesii* as the result of hypertrophy and hyperplasia. A more likely function of these responses in Artemisia tilesii and other species may be to reduce intercellular space in mesophyll layers thereby reducing the ability of acid solutions to penetrate nearby healthy tissue.

The results of the histological work indicated that lesion development is three-dimensional. Up to this point, lesion development has been considered as occurring in a vertical plane from the adaxial epidermis toward the interior of the leaf. Lesion development was most advanced at the center of the lesion and progressively earlier stages of injury occurred nearer to the lesion edge. This is consistent with an increase in depth of cellular damage towards the center. The greater depth of injury at the center of the lesion probably relates to both the longer time of exposure to acidic solutions as the droplets dry and the increase in hydrogen ion concentration as rain droplets evaporate on the leaf surface. Adjacent to damaged and collapsed cells at the centre of severe lesions, there is a region of extensive hyperplasia and hypertrophy which separates the severely injured tissue from healthy tissue. As mentioned, this may prevent lesions from becoming more extensive in species in which these responses occur.

The present study, and the earlier work by Evans, have shown that simulated acid rain affects leaf surface tissues initially and eventually injures more internal cells. In contrast, investigations have shown that gaseous pollutants initially affect mesophyll cells and the abaxial epidermis (Solberg and Adams, 1956; Shriner and Lacasse, 1969; Pell and Weissberger, 1976; Swieki et al, 1982). Macroscopic injury symptoms produced by acid rain can resemble those produced by some gaseous air pollutants. The ability to distinguish injury symptoms of acid rain from those of gaseous pollutants on the basis of histological investigation could provide a valuable tool for assessing the cause of injury to vegetation in the field. The importance of histological responses in sensitivity of species to acidic rain has been recognized, but the significance of these histological responses in determining plant response has not received sufficient emphasis in research.

Effects of acidified solutions on plant growth were also examined. Foliar chlorophyll concentrations were significantly reduced at pH 2.0, but were highest in the pH 3.0 treatment. There was a shift in the ratio of chlorophyll *a*:*b* at pH 2.0 suggesting chlorophyll *a* is more sensitive to acid than chlorophyll *b*. Sheridan and Rosenstreter (1973) reported chlorophyll *a* was affected to a greater extent than chlorophyll *b* in the moss *Tortula ruralis* in those acidic rain treatments which caused a reduction in chlorophyll concentration. The finding that chlorophyll was higher at pH 3.0 than at pH 5.6 is in agreement with some previous results (Ferenbaugh, 1976; Irving and Miller, 1977; Johnston *et al*, 1982). Hindawi *et al* (1980), on the other hand, reported an 8% reduction in chlorophyll content at pH 3.0, and greater reductions at pH 2.5 (15%) and pH 2.0 (31%). The higher chlorophyll levels observed at pH 3.0 suggest that a positive growth response occurs in some species as a result of sulfur or nitrogen inputs in the rain.

No significant growth reduction was observed in any of the acidic treatments except pH 2.0, where root biomass was reduced by 50 - 70%. Despite the enormous reduction in root weight, no effect on shoot weight occurred from the pH 2.0 treatment. Rainfall with a pH of 2.0

is well below the current levels of acidity observed in rainfall. The high sensitivity of roots compared to shoots is viewed as an indirect effect of the acid solutions rather than a direct effect, since plastic lids prevented acidic solutions from coming in contact with the roots. This suggests a shift in photosynthate allocation, resulting in reduced energy available for root growth. Ferenbaugh (1976) reported a reduction in shoot and root biomass in *Phaseolus vulgaris* at pH 2.0. Root biomass was more severely affected than was biomass of shoots. The sensitivity of roots observed in acid rain treatments is consistent with an investigation of the effect of simulated acid rain on crop yield of 28 species which concluded that root crops were the group most affected by acid rain (Lee *et al*, 1981). In contrast to the results for *Artemisia tilesii*, many of these root crops exhibited reductions in yield at pH values between 3.0 and 4.0, and in some cases in the absence of visible foliar damage. In cases where root reductions take place relative to shoots, energy for shoot repair of damage may be at the expense of root growth.

An increase in the dry weight of roots and shoots of *Artemisia tilesii* clones was observed between pH 3.5 and 3.0. Though not statistically significant, this is consistent with the increase in foliar chlorophyll content observed at pH 3.0. Beneficial effects of high nitrate and sulphate inputs through rainwater were probably responsible. Jacobson *et al* (1980) obtained the best growth of lettuce by treatment with simulated acidic rain at pH 3.2 containing high sulphate:nitrate ratios. The high nitrate pH 3.2 treatments were not significantly different in productivity from the pH 5.7 controls. Wood and Bormann (1974) reported that productivity of white pine seedlings was significantly increased as concentrations of H_2SO_4, HNO_3, and HCl were increased in artificial rain, adjusted to pH's as low as 2.3. The data of Raynal _et al_ (1982) showed that sugar maple seedlings exposed to pH 3.0 throughfall grew better than those at pH 5.2 when no supplemental nutrients were supplied. When nutrient solution was added to the soil, differences in seedling growth disappeared. These studies indicate that sulphur and nitrogen inputs in precipitation are important in the response of vegetation to acid precipitation. They also suggest that more attention should be given to soil nutrient status and plant nutrient requirements in simulated acid rain experiments in the future. Ideally plants should be grown in soils from their natural environment, which may be covered with a layer of leaf litter if this occurs. While nitrogen and sulphur in rainwater may be beneficial to the growth of plants in the short-term in the pH 4.0 to 3.0 range, productivity may be decreased in the long run as a result of damage to leaf tissues. Long-term studies of productivity under acid-stressed conditions are necessary.

At the present time, there is insufficient information to allow evaluation of the effects of ambient and/or anticipated levels of acidity on plant productivity. Greenhouse experiments using potted plants have yielded a number of hypotheses which must now be tested in the field situation. Accurate determination of the effects of acid precipitation will also depend on a better knowledge of the characteristics of the rain event (i.e. flucuating acidity levels, duration, intensity, frequency, droplet size) and of the environment (i.e. humidity, sunlight, temperature, wind speed) that affect plant response.

As a result of increased rainfall acidity, alterations occurred in the rate of nutrient leaching from Artemisia tilesii leaves. Plants which were exposed to pH 2.0 rainfall for 4 weeks showed significant losses in foliar calcium, magnesium and potassium when compared to the pH 5.6 control, although there was no effect apparent above the pH 2.0 treatment. Sodium concentrations were not affected. Other studies have examined leaching of nutrients caused by exposure to acidic mists (Wood and Bormann, 1975; Fairfax and Lepp, 1975, 76; Hindawi et al, 1977; Horntvedt, 1977, 78; Evans et al, 1981a). All of these studies reported leaching from rain solutions at less acidic levels. Loss of nutrients was demonstrated even without any visible damage to the foliage. In order to understand the mechanism responsible for resistance to leaching in Artemisia tilesii further studies are necessary, in which leachate from leaves is analysed in addition to the foliar analyses. Two explanations are possible: (1) leaf cells are less permeable to ions or (2) rates of nutrient uptake in acid-stressed plants are higher and compensate for nutrients that are lost. Since visible tissue injury was evident at pH 2.5 and some cellular damage occurred at pH 3.0, one would suspect that losses of nutrients are occurring to plants from these treatments, which can be replaced.

The relative susceptibility of ions in leaves to leaching has produced contradictory results. In tobacco leaves, calcium leached faster from foliage exposed to simulated rain of pH 3.0 than from foliage exposed to pH 6.7, whereas the amount of potassium leached was greater from leaves exposed to pH 6.7 simulated rain than from leaves exposed to pH 3.0. Little change occurred in levels of magnesium lost (Fairfax and Lepp, 1975). Evans et al (1981a) have also reported that more potassium was leached from foliage of pinto beans exposed to a control rainfall (pH 5.6) than from foliage treated with simulated rain at pH levels of 2.7, 2.9 and 3.1. Greater amounts of calcium, nitrate and sulphate were leached from the pinto beans by acidic rainfall, while the amounts of ammonium, magnesium and zinc were the same at all of the pH levels. Reductions in foliar concentrations of nitrogen, calcium, phosphorous and magnesium occurred when bush beans were exposed to an acidic mist, whereas potassium concentrations were not affected (Hindawi _et al_, 1977). These results contrast with those for _Artemisia tilesii_ and also with reports from bean and maple seedlings (Wood and Bormann, 1975) which showed significant losses of potasssium, magnesium and calcium in response to acidity, with no affect to sodium levels. A wide range of other plant metabolites, including amino acids, carbohydrates and growth regulators are also removed from plants by rain (Tukey, 1970; 1975). The effect of simulated acid rain on leaching of other important substances in leaves besides nutrients, should be considered in future studies.

Observations made at the Smoking Hills, that strongly acidic rain droplets falling on leaves of _Artemisia tilesii_ were quickly neutralized, suggested that the ability of _A. tilesii_ to buffer acidic droplets might be important in its resistance to acidic rain. Similarly, a lack of buffering ability might account for the sensitivity of other species to this pollutant. This hypothesis was tested by applying acidic droplets to the surface of leaves of _Artemisia tilesii_, as well as to leaves of _Phaseolus vulgaris_ and _Spinacea oleracea_ which are more sensitive to simulated acid rain.

Although neutralization of acidic droplets occurred on the leaves of all three species, buffering capacity of leaves was significantly higher in the two populations of Artemisia tilesii. The ability to buffer acidic droplets correlated with the amount of injury observed on the foliage. Spinacea, which exhibited the most foliar injury, had the least ability to buffer acidic droplets. While leaves of spinach showed an initial rapid neutralization of droplets at pH 3.0 and 2.5, this was not sustained over the 75 minutes it took for the droplets to dry. Variation in the buffering capacity was observed between leaves on the same plant and even between different areas on the same leaf. The differences in the quantity of alkaline substances released from different areas of the leaf is probably due to microtopographic features of the leaf surface, such as variations in wax quantities and constituents and perhaps proximity to veins. The region surrounding a secretory gland may also have a higher alkalinity. Nevertheless, the variation between leaves and within a leaf was smaller than the differences observed between the different species.

These results suggest that buffering by leaves may provide an explanation for differences in species sensitivity to acid rain. Further work is necessary, testing a greater number of species, before this can be accepted as a general phenomenon. Correlations between buffering capacity of needle homogenate and resistance to SO_2 damage have been reported (Grill and Härtell, 1972; Scholz and Reck, 1977) and between buffering capacity of needle homogenate and resistance to pH-changing fungi (Scholz and Stephan, 1974, 75). In SO_2 tolerant Picea abies, buffering capacity was suggested to be under genetic control (Scholz and Reck, 1977). Since SO_2 forms sulphurous acid upon coming in contact with plant cells, a common mechanism may operate externally and internally to buffer acidic precipitation and SO_2 generated acidity.

Neutralization of acidic droplets may be brought about when cations are leached from exchangeable pools or from salts deposited on the leaf surface. Leaching of cations involves exchange reactions on the leaf surface in which cations on exchange sites of the cuticle are exchanged by hydrogen from leaching solutions, and/or cations move directly from the translocation stream within the leaf into the leaching solution by diffusion and mass flow through areas devoid of cuticle (Mecklenburg et al, 1966).

The primary pathway for cation loss is probably through micropores in the cuticle. Schönherr and Bukovac (1970) demonstrated the existence of discrete areas where substances penetrated the cuticle based on the nature of mercury precipitates formed in the outer cell wall. The fact that cuticular pores were the sites of transcuticular penetration was clearly demonstrated in kinetic studies by McFarlane and Berry (1974). Mecklenburg et al (1966) have demonstrated that leaching is primarily a passive process such as diffusion. However, considerable quantities of cations may also be deposited onto the leaf surface from within by active processes such as guttation from hydathodes (Curtis, 1943), glandular trichomes (Ivanoff, 1963) and salt glands (Thomson, 1975).

The cations lost from leaves, whether by guttation or by leaching by rain, dew or mist, primarily constitute Na, K, Mg, Ca and Mn. Tukey et al (1966) have postulated that the leached cations combine with dissolved CO_2 in the leaching solution to form alkaline carbonates which either remain in the leaching solution or are deposited onto the leaf surface. Sometimes this deposition of material can be so severe that leaves become coated in a white crust. This is consistent with the characteristic alkaline nature of plant leachates and the fact that anions are not leached in amounts equivalent to cations. This also suggests that plants which characteristically contain a relatively high salt accumulation, or which are capable of rapidly mobilizing cations, would be more tolerant of acidic rain than those that do not. It emphasizes the risk of injury with rains of low pH and long duration as compared with rains of low pH and short duration. In acid rainfalls which last for several hours, nutrient levels would become depleted in the leaves and injury to the plant significantly increased because of loss of neutralizing ability. The length of the duration between rainfalls is also important to plant response by allowing the plant to replace nutrients in sufficient quantities to overcome leaching losses.

In these experiments more mature leaves were better at buffering the acidic droplets than young ones. This is consistent with reports that nutrients are in exchangeable forms in mature leaves and are more easily leached than in young growing tissue where nutrients are quickly metabolized within cells (Tukey _et_ _al_, 1958; Tukey, 1970b; Tukey, 1971). It may also be related to the wetting properties of the leaf, in that young leaves are hydrophobic and wetted with difficulty.

The quantities of cations released by leaves were significantly increased as the acidity of the droplets increased (Table 50). Approximately 4000 μeq l^{-1} H_3O^+ were neutralized by _Artemisia_ leaves one hour after droplets of pH 2.5 were applied, compared to 1000 μeq l^{-1} when the droplet pH was 3.0, 500 μeq l^{-1} when the droplet pH was 3.5, and only 2 μeq l^{-1} when the droplet pH was 5.6. This suggests that either there is a greater exchange capacity due to the increased [H^+] or that leaves respond by actively pumping cations to the leaf surface to regulate the pH environment at the leaf surface. Buffering capacity of the leaves of all three species used in these experiments was not adequate to overcome the amount of acidity delivered by droplets of pH 2.5. This indicates that there is a threshold pH between pH 3.0 and 2.5, beyond which the alkaline releases from the leaf surface do not significantly raise the rainwater pH. The measurements also showed that evaporation of very acidic droplets on the leaf surface can result in significantly lower pH values than the original droplet pH. After 75 minutes pH 2.5 rain droplets had a pH close to 2.0 on the leaf surface.

It is difficult to extrapolate the laboratory results to field conditions. In addition to exudations from the plant itself, soil particles and dust on the leaf surface may significantly buffer acidity in rain droplets or mist, particularly in understory and crop plants. On the other hand, nutrient concentrations in greenhouse plants are often higher than in plants growing out-of-doors (Tukey, 1970a). Studies are necessary to look at buffering capacity of plants under field conditions.

It has been demonstrated that plants can replace leached nutrients with nutrients from other parts of the plant (Tukey _et al_, 1958). One might speculate that the nutritional status of plants has an important bearing on their susceptibility to acid precipitation. In future, more attention might well be given to leaves as an active organ of excretion which tends to adjust the plant to its environment. This regulation functions at both the cellular and extracellular level.

SUMMARY AND CONCLUSIONS

Experiments were performed to determine the response of several clones of *Artemisia tilesii* to simulated acid rain. In some experiments *Phaseolus vulgaris* and *Spinacea oleracea* were also used to provide a comparison with the response of *A. tilesii*. This species was selected for study because its foliage showed a high tolerance to acidic droplets and SO_2 at the Smoking Hills, N.W.T. Thus, it provided an excellent species in which one could examine possible characteristics that may confer tolerance to acidic precipitation in plants. Of particular interest in this study was the possibility that buffering capacity of leaves might be important in the resistance of *A. tilesii* to acidic rain, since preliminary observations at the Smoking Hills showed that acidic droplets on leaves of *A. tilesii* were neutralized.

Results from a detailed study comparing the response of clones from the Smoking Hills population with the responses of clones from an unpolluted (control) population in Alaska have shown that clones from the two populations respond similarly to simulated acid rain. Both populations showed a high tolerance. This indicates that the resistance of *Artemisia tilesii* is an inherent characteristic of this species, rather than the result of selection under acidic conditions at the Smoking Hills. It is cautioned that only a small number of clones from each population were tested, so that further work is necessary to confirm these results. Variation in the percentage of leaves which were injured for the six Smoking Hills clones was related closely to morphological charactersitics of these clones. Plant stature, leaf size and shape, the amount of leaf dissection and leaf orientation were critical features in plant response. While morphological features showed a strong correlation with the percentage of leaves injured, there was no similar relationship with the percent of leaf area injured.

Plant response varies with characteristics of the rain event itself. The pattern of response to rainfall duration and frequency is complex. Injury was increased significantly with rainfalls of longer duration at pH 2.0, but not at pH 3.0, whereas injury was greater on plants sprayed daily than on plants sprayed every other day at pH 3.0, but not at pH 2.0. It is considered that plant response to duration and frequency of rainfall may be related to the buffering capacity of leaves. Injury will increase when leaves no longer have the capacity to buffer acidic droplets on the leaf surface. For rain events of low pH, buffering capacity is probably exhausted very rapidly.

Cellular injury from acidic rain was more extensive than macroscopic injury and occurred when rainfall was less acidic. Observations of cellular injury in the absence of macroscopic injury, indicates that "invisible injury" from acidic precipitation may occur. These results showed that visual estimates of leaf surface injury may significantly underestimate the damage to leaves from acid rain. Anatomical investigations could prove to be a more sensitive indicator of acid rain injury to vegetation from exposure to natural rain or mist.

SEM examination revealed significant differences in stomatal aperture size between plants treated with acidified rain solutions (pH 2.5) and those treated with rain of pH 5.6. Simulated acid rain interfered with the ability of guard cells to function normally in stomatal closure. This may increase the susceptibility of acid-injured foliage to infection by epiphytic plant pathogens, drought stress and gaseous air pollutants, as well as to acidic components in subsequent rainfall.

A detailed study of the histological responses of leaves of A. tilesii to acidic rain indicates that injury begins in the adaxial epidermis and eventually affects more internal cells. In most instances, cells of the adaxial epidermis collapsed before any injury occurred in the mesophyll tissues. A second pathway of lesion development, which was less common, was that of hypertrophy (abnormal cell enlargement) and hyperplasia (abnormal cell division) in mesophyll tissues without the collapse of overlying epidermal cells. However, when this occurred cells of the adaxial epidermis were stained heavily, clearly indicating cellular injury had occurred. The mechanism for this response is not known. Perhaps the permeability of epidermal cells was altered by acidic solutions which allowed acidic rainwater to penetrate mesophyll tissues. Damage to leaf tissues was most severe at the lesion center and diminished towards the periphery. Severely damaged tissue at the center of the lesion was separated from uninjured tissue by a region of extensive cell hypertrophy and hyperplasia. Intercellular space was reduced considerably by these responses, suggesting that they may be important in preventing the spread of injury into adjacent healthy tissue. The importance of histological responses in sensitivity of species to acidic rain has been recognized, but has not received sufficient emphasis in research.

Dry weight was reduced only in the pH 2.0 treatment. An increase in root and shoot weight, though not statistically significant, was observed at pH 3.0 and 3.5 for all of the clones. A similar increase in the chlorophyll content of plants treated with pH 3.0 rainfall suggests that growth may be stimulated by sulphate and nitrate additions in the rainwater. Leaching of significant quantities of calcium, magnesium and potassium occurred at pH 2.0 compared to control rainfall (pH 5.6). No change was observed in foliar cation levels above pH 2.0, even though considerable foliar injury occurred to plants exposed to rainfall at pH 2.5. These experiments were carried out over a relatively short time period. Further studies are necessary to determine the long term effects of acid precipitation on plant productivity and foliar nutrient content.

Artemisia tilesii differed markedly in buffering capacity from *Spinacea oleracea* and *Phaseolus vulgaris*. Acidic droplets placed on leaves of *A. tilesii* were neutralized more than droplets on either *P. vulgaris* or *S. oleracea* leaves. The lowest buffering capacity was in spinach plants, which are also more sensitive to injury from simulated acid rain. Mature leaves had a higher buffering capacity than young leaves. Much larger quantities of alkaline substances were released from leaves when the acidity of droplets increased. Neutralization of acidic droplets may be brought about when cations are leached from exchangeable pools or from salts actively deposited on the leaf surface. Many more physiological aspects of this resistance mechanism remain to be investigated, particularly the way in which cations are released onto the leaf surface, rates of release from species varying in sensitivity to simulated acid rain, the effects of foliar cation content on buffering capacity and the effect of soil nutrient status on buffering capacity. The buffering capacity of

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plants might be used in crop breeding work as an indirect method of selection for resistance to acid precipitation.

This study has provided information on which characteristics of a plant are important in the sensitivity to acidic components in rainwater. Knowledge of the plant features which impart resistance is extremely important since it could then be used to predict which tree, understory and crop species are especially at risk from acid precipitation.

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Appendix 1. The effect of rainfall duration and frequency on the percent of Artemisia tilesii (Smoking Hills) leaves injured by simulated acid rain.
(Each value represents the mean of six plants)

Rain pH	Spray freq.	Spray duration (mins.)	Percent leaves injured					
			1 spray		5 sprays		10 sprays	
			$\overline{x}$	(SE)	$\overline{x}$	(SE)	$\overline{x}$	(SE)
2.0	every day	2.5	8.7	(2.8)	77.2	(4.4)	94.5	(2.3)
		5	29.3	(6.3)	90.2	(3.6)	99.3	(0.6)
		10	21.8	(5.6)	92.5	(2.3)	97.3	(1.7)
		20	21.7	(7.9)	97.8	(2.2)	99.5	(0.5)
	every other day	2.5	-	-	98.7	(1.0)	100.0	(0.0)
		5	-	-	99.2	(0.5)	100.0	(0.0)
		10	-	-	100.0	(0.0)	100.0	(0.0)
		20	-	-	99.2	(0.8)	100.0	(0.0)
3.0	every day	2.5	0.0	(0.0)	6.0	(3.2)	74.5	(10.5)
		5	0.5	(0.5)	3.8	(3.3)	76.2	(5.6)
		10	0.0	(0.0)	8.0	(2.0)	59.2	(3.9)
		20	0.0	(0.0)	39.2	(16.1)	66.2	(6.9)
	every other day	2.5	-	-	16.5	(2.6)	25.2	(5.8)
		5	-	-	14.5	(5.9)	31.3	(10.5)
		10	-	-	13.7	(3.8)	22.2	(6.3)
		20	-	-	14.0	(3.2)	24.7	(3.5)
5.6	every day	2.5	No injury					
		5						
		10						
		20						
	every other day	2.5	No injury					
		5						
		10						
		20						

Appendix 2. Leaf injury to six Smoking Hills *Artemisia* clones over a four week period.
(Each value represents the mean of ten plants)

Rain pH	Clone No.	Percent leaves injured ($\bar{x}$ ± S.D.)				
		1 Spray (1 day)	3 Sprays (1 week)	6 Sprays (2 weeks)	9 Sprays (3 weeks)	12 Sprays (4 weeks)
2.0	3	81.2± 7.1	97.4± 3.4	99.0± 1.8	99.6± 1.3	100.0± 0.0
	5	71.9±11.2	90.5± 6.5	96.9± 2.8	99.2± 1.4	99.6± 0.8
	8	72.6± 9.6	91.7± 6.1	97.7± 2.2	99.3± 1.2	99.5± 1.1
	9	85.2±10.5	99.4± 1.3	99.3± 1.5	100.0± 0.0	100.0± 0.0
	11	75.0±12.8	94.6± 4.1	96.7± 4.1	98.6± 2.6	98.8± 2.3
	12	53.2±13.5	85.3± 9.6	92.9± 5.3	96.6± 3.9	97.1± 3.5
Grand Mean		73.2±14.6	93.8± 5.7	97.1± 3.7	98.9± 2.3	99.2± 2.0
2.5	3	59.3±12.5	83.9±20.6	90.2± 7.5	96.4± 2.5	97.7± 2.7
	5	45.5± 4.6	69.8±10.0	84.8± 7.3	90.5± 6.7	95.1± 3.8
	8	55.8±20.1	77.8± 6.4	87.4± 5.7	87.7± 4.1	92.0± 4.4
	9	68.8± 8.8	87.2± 7.6	92.7± 4.1	96.1± 3.0	97.4± 2.7
	11	49.5±14.5	68.1±13.3	82.5± 7.6	86.8± 7.0	84.0± 7.9
	12	43.3±16.9	63.2±13.3	77.6± 7.4	82.1± 9.3	87.1± 6.6
Grand Mean		53.7±16.0	74.1±12.7	85.9± 8.2	89.9± 7.7	92.2± 7.1
3.0	3	0.0± 0.0	17.6±12.7	33.7±17.7	54.8±14.3	80.1±14.7
	5	0.0± 0.0	6.9± 5.0	21.0±15.7	40.2±14.8	54.5±14.1
	8	0.0± 0.0	9.4± 7.9	24.2± 8.3	38.5±14.8	56.7±16.5
	9	0.0± 0.0	19.5±15.6	36.6±18.0	58.6±17.8	83.0±16.7
	11	0.0± 0.0	1.4± 3.5	6.3± 7.5	12.3± 6.6	24.4±10.9
	12	0.0± 0.0	7.0± 8.3	22.5±24.1	26.1±21.9	36.4±20.6
Grand Mean		0.0± 0.0	10.3±11.3	24.1±18.4	38.4±22.0	55.9±26.2
4.0		No injury to any of the six clones				
5.6		No injury to any of the six clones				

NOTE: Statistical analyses of these injury values (after an arcsin $\sqrt{}$ transformation) are shown in Tables 17 and 18.

Appendix 3. Percentage of leaves injured for Artemisia clones from the Smoking Hills and from Alaska.
(Values are the mean of 14 plants ± 1 S.D.)(Plants were sprayed twice weekly for a 20 minute period.)

Rain pH	Population and Clone no.	Number of 20 minute exposures						
		1 $\bar{x}$ ± S.D.	2 $\bar{x}$ ± S.D.	4 $\bar{x}$ ± S.D.	6 $\bar{x}$ ± S.D.	8 $\bar{x}$ ± S.D.	10 $\bar{x}$ ± S.D.	12 $\bar{x}$ ± S.D.
2.5	Smoking Hills 8	30.9 ± 3.9	51.7 ± 20.4	84.0 ± 7.8	88.1 ± 4.5	86.1 ± 5.9	84.4 ± 6.3	87.8 ± 4.7
	9	46.3 ± 14.3	73.1 ± 16.6	85.6 ± 8.1	89.7 ± 5.3	83.4 ± 6.2	79.5 ± 6.7	82.8 ± 6.5
	11	16.9 ± 14.8	30.5 ± 10.7	77.8 ± 9.8	86.1 ± 6.6	84.9 ± 6.3	89.1 ± 3.4	92.5 ± 3.0
	Overall	31.0 ± 18.5 n=41	51.8 ± 23.7 n=40	82.5 ± 9.0 n=40	87.9 ± 5.6 n=41	84.8 ± 5.9 n=41	84.4 ± 6.8 n=40	87.8 ± 6.2 n=41
	Alaska 2	1.9 ± 3.7	11.4 ± 11.2	58.4 ±15.0	80.3 ± 7.5	80.6 ±10.0	86.1 ± 8.9	89.3 ± 9.2
	3	10.6 ± 11.2	15.1 ± 9.4	81.8 ±13.9	91.4 ± 5.5	90.0 ± 4.1	98.4 ± 2.0	98.9 ± 2.0
	5	3.7 ± 7.5	19.6 ± 22.8	56.2 ±15.7	91.6 ± 5.9	85.7 ± 7.9	87.7 ± 5.6	92.0 ± 5.0
	Overall	5.7 ± 8.9 n=36	14.7 ± 14.3 n=36	66.5 ±18.7 n=35	87.4 ± 8.3 n=36	85.5 ± 8.5 n=36	91.3 ± 8.4 n=36	93.7 ± 7.4 n=36

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Appendix 3 (continued)

Rain pH	Population and Clone no.	Number of 20 minute exposures						
		1 $\bar{x}$ ± S.D.	2 $\bar{x}$ ± S.D.	4 $\bar{x}$ ± S.D.	6 $\bar{x}$ ± S.D.	8 $\bar{x}$ ± S.D.	10 $\bar{x}$ ± S.D.	12 $\bar{x}$ ± S.D.
3.0	Smoking Hills 8	0.7 ± 2.7	1.3 ± 3.7	1.0 ± 1.8	10.3 ± 9.7	18.6 ±23.5	15.1 ±15.9	16.9 ±20.8
	9	2.8 ± 4.8	2.0 ± 3.8	0.8 ± 1.6	16.7 ± 7.9	19.7 ± 6.4	56.8 ±16.9	60.3 ±13.6
	11	0.0 ± 0.0	0.2 ± 0.8	0.0 ± 0.0	1.6 ± 4.2	2.8 ± 3.7	2.9 ± 6.5	1.1 ± 2.4
	Overall	1.2 ± 3.3 n=40	1.1 ± 3.0 n=41	0.6 ± 1.5 n=40	9.4 ± 9.7 n=41	14.0 ±16.3 n=38	23.3 ±26.4 n=40	24.4 ±28.4 n=40
	Alaska 2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 1.7	2.5 ± 3.0	1.2 ± 1.9
	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 2.2	0.8 ± 2.0	0.9 ± 1.4	0.9 ± 1.8
	5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 2.8	6.9 ± 9.2	9.1 ± 6.3	6.2 ± 7.1
	Overall	0.0 ± 0.0 n=36	0.0 ± 0.0 n=36	0.0 ± 0.0 n=35	0.9 ± 2.2 n=37	2.1 ± 5.1 n=36	3.2 ± 4.6 n=37	2.3 ± 4.4 n=36
3.5	No injury to any of the clones							
4.0	No injury to any of the clones							
5.6	No injury to any of the clones							

Appendix 4. Percent leaf area injured for Artemisia clones from the Smoking Hills and from Alaska.
(Values are means for seven plants ± 1 S.D.)[a]

pH	Population	Clone	18 sprays (9 weeks) $\bar{x}$ ± S.D.	24 sprays (12 weeks) $\bar{x}$ ± S.D.
2.5	Smoking Hills	9	8.3 ± 4.9	10.0 ± 6.1
		8	4.6 ± 2.7	3.9 ± 2.4
		11	3.2 ± 1.9	3.2 ± 1.9
	Alaska	3	3.9 ± 2.4	2.1 ± 0.9
		2	2.5 ± 0.0	2.5 ± 0.0
3.0	Smoking Hills	9	1.7 ± 1.3	1.7 ± 1.3
		8	0.7 ± 1.2	0.0 ± 0.0
		11	0.0 ± 0.0	0.0 ± 0.0
	Alaska	3	0.4 ± 0.9	0.0 ± 0.0
		2	0.0 ± 0.0	0.4 ± 0.9
3.5		No injury occurred to any of the clones		
4.0		No injury occurred to any of the clones		
5.6		No injury occurred to any of the clones		

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[a] Percent leaf area injured was scored on fully expanded leaves using 10 categories: 0%, 1 - 5%, 6 - 10%, 11 - 25%, 26 - 50%, 51 - 75%, 76 - 90%, 91 - 95%, 96 - 99%, 100%.

Appendix 5. Expected and measured frequencies of lesions occurring over vascular tissues.

CALCULATIONS OF THE EXPECTED FREQUENCY OF LESIONS OVER VASCULAR TISSUE

Abbreviations: VB = vascular bundle

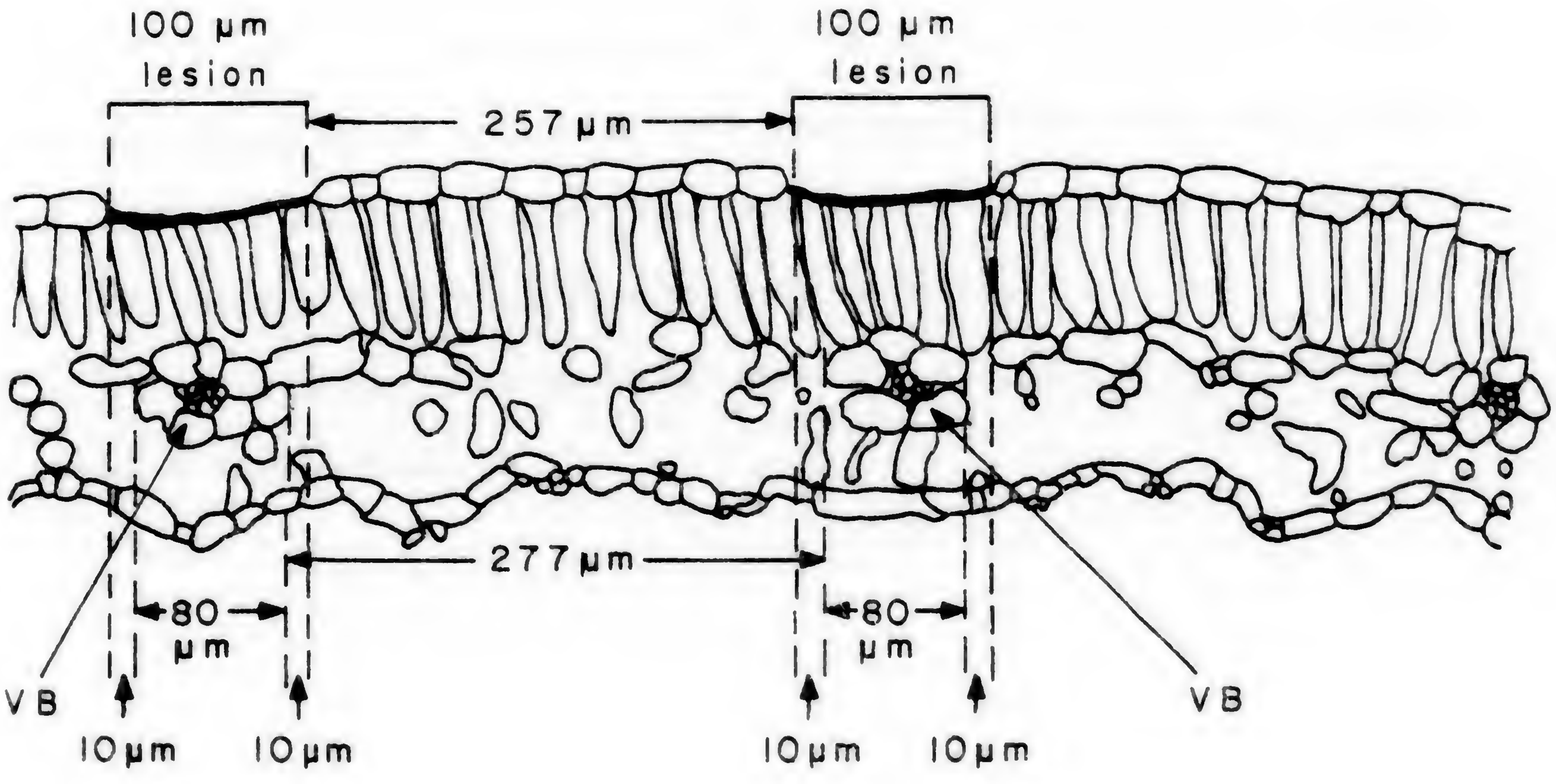
(1) Smoking Hills leaves

Mean distance between vascular bundles = 276.9 µm

Mean width of vascular bundles = 79.4 µm

Lesions up to 100 µm in diameter (i.e. small sized lesions) would extend 100 µm - 79 µm (width of VB) = 21 µm into non-vascular tissue and be classified as occurring over vascular tissue. This leaves 276.9 µm - 21 µm (lesions extend 10.5 µm out from either side of a VB) = 256.9 µm of non-vascular tissue between vascular bundles.

Thus, there is 256.9 µm of non-vascular tissue for every 100 µm of vascular tissue zone as seen in the diagram below:



Appendix 5 (continued).

CALCULATED VALUE: 2.57 µm of non-vascular tissue exists for every 1 µm of vascular zone tissue. This mean that 2.57 times as many lesions should occur in non-vascular tissue as do in vascular tissue.

ACTUAL VALUE FOR SMOKING HILLS LEAVES: (based on experiment 1 data given in Table 28, for which 109 lesions in the < 100 µm size category were scored).

65% of lesions occurred in non-vascular tissue giving a ratio of 1.86:1 in non-vascular:vascular tissue.

(2) Alaskan leaves

Mean distance between vascular bundles = 264.3 µm

Mean width of vascular bundles = 63.0 µm

Lesions up to 100 µm in diameter (i.e. small sized lesions) would extend 100 µm - 64 µm (width of VB) = 36 µm into non-vascular tissue and be classified as occurring over vascular tissue. This leaves 264.3 µm - 36 µm (lesions extend 18 µm from either side of a VB) = 228.3 µm of non-vascular tissue between vascular bundles.

Thus there is 228.3 µm of non-vascular tissue for every 100 µm of vascular tissue zone.

Appendix 5 (continued).

CALCULATED VALUE: 2.28 µm of non-vascular tissue exists for every 1 µm of vascular zone tissue, which means that 2.28 times as many lesions should occur in non-vascular tissue as do in vascular tissue.

ACTUAL VALUE FOR ALASKAN LEAF LESIONS: (based on experiment 2 data given in Table 28, for which 131 lesions in the < 100 µm size category were scored).

63% of lesions occurred in non-vascular tissue giving a ratio of 1.70:1 lesions occurring in non-vascular:vascular tissue.

INTERPRETATION: A higher than expected frequency of lesions in the initial stage of development (<100 µm diameter) occurred over vascular tissue indicating that this is a preferential site for lesion development.

Appendix 6. Measurements of cell dimensions in leaves from acidic and control rainfall treatments. (Values represents the mean of 10 cells measured for each of four leaves).

	pH 5.6				pH 3.0				pH 2.5			
Smoking Hills cell height (µm)	1 spray $\bar{x}$ (SE)	2 sprays $\bar{x}$ (SE)	4 sprays $\bar{x}$ (SE)	8 sprays $\bar{x}$ (SE)	1 spray $\bar{x}$ (SE)	2 sprays $\bar{x}$ (SE)	4 sprays $\bar{x}$ (SE)	8 sprays $\bar{x}$ (SE)	1 spray $\bar{x}$ (SE)	2 sprays $\bar{x}$ (SE)	4 sprays $\bar{x}$ (SE)	8 sprays $\bar{x}$ (SE)
upper epidermis	30.53 (1.23)	34.87 (3.57)	34.67 (2.19)	34.18 (3.14)	30.83 (2.90)	35.36 (3.34)	16.65 (9.69)	25.61 (8.95)	5.71 (5.71)	6.11 (6.11)	5.91 (5.91)	0.00 (0.00)
palisade (1) parenchyma	63.53 (1.37)	62.55 (4.59)	70.13 (8.90)	74.27 (1.99)	60.77 (4.54)	58.51 (8.58)	64.02 (7.29)	63.43 (7.51)	8.57 (8.57)	25.90 (15.28)	39.40 (13.52)	20.26 (11.90)
palisade (2) parenchyma	46.59 (4.59)	53.98 (5.12)	52.50 (6.96)	61.07 (5.18)	48.36 (2.82)	48.00 (1.52)	54.25 (2.05)	49.45 (5.74)	5.81 (5.81)	20.09 (12.18)	61.08 (7.90)	31.45 (18.99)
lower spongy parenchyma	23.54 (2.53)	23.34 (2.74)	21.77 (1.02)	31.72 (2.21)	23.93 (2.39)	28.56 (1.56)	29.55 (4.84)	23.93 (2.67)	18.02 (1.40)	23.44 (3.66)	27.58 (2.74)	27.87 (2.90)
lower epidermis	18.12 (0.96)	20.78 (1.49)	20.59 (1.63)	23.84 (0.61)	19.99 (1.20)	19.70 (2.27)	19.99 (1.13)	19.90 (1.68)	12.31 (4.33)	17.83 (2.89)	24.23 (2.95)	15.46 (5.18)

Continued

Appendix 6 (Continued).

Smoking Hills cell width (µm)	pH 5.6				pH 3.0				pH 2.5			
	1 spray $\bar{x}$ (SE)	2 sprays $\bar{x}$ (SE)	4 sprays $\bar{x}$ (SE)	8 sprays $\bar{x}$ (SE)	1 spray $\bar{x}$ (SE)	2 sprays $\bar{x}$ (SE)	4 sprays $\bar{x}$ (SE)	8 sprays $\bar{x}$ (SE)	1 spray $\bar{x}$ (SE)	2 sprays $\bar{x}$ (SE)	4 sprays $\bar{x}$ (SE)	8 sprays $\bar{x}$ (SE)
upper epidermis	47.87 (2.64)	57.52 (7.59)	48.66 (7.53)	42.55 (3.08)	54.08 13.60)	50.53 (2.79)	22.95 (13.45	31.42 11.03)	8.37 (8.37)	9.46 (9.46)	6.99 (6.99)	0.00 (0.00)
palisade (1) parenchyma	25.61 (2.79)	29.84 (2.50)	24.62 (2.22)	25.02 (1.35)	24.13 (0.65)	27.19 (1.19)	26.50 (3.52)	22.06 (1.95)	4.92 (4.92)	12.02 (7.46)	27.58 (11.00)	24.13 (14.21)
palisade (2) parenchyma	33.29 (2.71)	30.93 (1.78)	28.37 (3.39)	26.30 (1.22)	25.81 (0.86)	30.93 (0.93)	29.75 (2.85)	25.22 (1.50)	6.99 (6.99)	11.82 (7.12)	38.43 (6.50)	21.22 (12.26)
lower spongy parenchyma	26.89 (1.02)	24.62 (2.22)	22.06 (1.66)	25.12 (1.03)	29.75 (2.06)	27.38 (2.46)	26.40 (5.26)	22.16 (1.75)	20.09 (2.43)	20.09 (2.49)	28.66 (5.11)	23.44 (1.04)
lower epidermis	28.56 (2.46)	30.53 (2.34)	27.87 (3.59)	32.11 (1.60)	29.16 (1.39)	22.75 (2.42)	30.04 (0.90)	26.30 (2.58)	14.77 (5.09)	24.13 (2.55)	28.66 (4.27)	18.71 (6.29)

Continued

Appendix 6 (Continued).

Alaska cell height (μm)	pH 5.6				pH 3.0				pH 2.5			
	1 spray $\bar{x}$ (SE)	2 sprays $\bar{x}$ (SE)	4 sprays $\bar{x}$ (SE)	8 sprays $\bar{x}$ (SE)	1 spray $\bar{x}$ (SE)	2 sprays $\bar{x}$ (SE)	4 sprays $\bar{x}$ (SE)	8 sprays $\bar{x}$ (SE)	1 spray $\bar{x}$ (SE)	2 sprays $\bar{x}$ (SE)	4 sprays $\bar{x}$ (SE)	8 sprays $\bar{x}$ (SE)
upper epidermis	14.68 (0.86)	16.35 (0.73)	15.46 (0.56)	19.21 (1.02)	6.60 (2.80)	14.58 (0.16)	5.35 (2.65)	19.01 (0.46)	8.57 (3.52)	10.74 (4.40)	0.00 (0.00)	8.67 (5.00)
palisade (1) parenchyma	39.89 (3.58)	43.73 (0.48)	47.38 (3.86)	59.10 (1.99)	39.01 (3.96)	41.96 (3.19)	24.62 10.13)	53.68 (5.41)	26.10 (8.74)	41.96 (4.03)	11.13 (11.13)	21.83 (12.81)
upper spongy parenchyma	22.56 (0.79)	21.37 (2.58)	28.76 (2.45)	20.68 (1.46)	21.01 (4.10)	20.29 (1.00)	18.61 (6.33)	26.89 (5.55)	14.48 (4.88)	16.55 (3.74)	14.38 (8.74)	18.07 (10.74)
lower spongy parenchyma	14.77 (2.68)	15.56 (2.46)	19.31 (0.58)	23.25 (3.18)	15.96 (1.99)	19.11 (0.67)	15.42 (5.74)	30.14 (2.66)	12.41 (4.32)	14.97 (0.32)	10.05 (5.80)	24.31 (8.75)
lower epidermis	9.46 (0.58)	12.41 (0.52)	11.13 (0.86)	12.61 (0.42)	10.05 (0.34)	11.43 (0.80)	9.26 (3.19)	11.92 (0.19)	9.06 (3.03)	10.93 (0.79)	7.29 (4.22)	2.56 (2.56)

Continued

Appendix 6 (Continued).

Alaska cell width (μm)	pH 5.6				pH 3.0				pH 2.5			
	1 spray $\bar{x}$ (SE)	2 sprays $\bar{x}$ (SE)	4 sprays $\bar{x}$ (SE)	8 sprays $\bar{x}$ (SE)	1 spray $\bar{x}$ (SE)	2 sprays $\bar{x}$ (SE)	4 sprays $\bar{x}$ (SE)	8 sprays $\bar{x}$ (SE)	1 spray $\bar{x}$ (SE)	2 sprays $\bar{x}$ (SE)	4 sprays $\bar{x}$ (SE)	8 sprays $\bar{x}$ (SE)
upper epidermis	22.95 (2.03)	24.13 (4.40)	20.59 (1.76)	26.59 (0.97)	14.97 (4.08)	25.31 (0.90)	5.32 (5.32)	26.30 (1.63)	11.92 (7.21)	13.99 (8.30)	0.00 (0.00)	12.90 (7.55)
palisade (1) parenchyma	17.73 (0.83)	19.01 (0.91)	19.11 (0.94)	15.66 (0.54)	18.91 (4.57)	16.94 (1.71)	13.79 (5.39)	15.99 (1.26)	10.64 (3.56)	14.08 (1.67)	3.74 (3.74)	10.47 (6.26)
upper spongy parenchyma	23.74 (3.14)	25.90 (1.11)	29.06 (5.03)	27.68 (1.70)	28.70 (3.02)	25.71 (1.38)	20.39 (7.91)	28.27 (2.35)	20.59 (7.01)	22.26 (5.23)	15.37 (9.28)	16.69 (9.65)
lower spongy parenchyma	23.15 (3.32)	20.19 (2.87)	24.53 (1.88)	20.78 (0.76)	19.40 (3.17)	22.36 (2.02)	18.53 (6.90)	22.36 (1.59)	16.15 (5.49)	20.29 (1.78)	10.54 (6.10)	25.82 (9.12)
lower epidermis	17.43 (2.35)	16.74 (1.71)	16.84 (1.13)	18.22 (2.02)	12.90 (0.61)	17.14 (2.77)	12.11 (4.33)	16.74 (0.92)	13.59 (4.54)	16.84 (1.42)	8.67 (5.10)	4.43 (4.43)

Appendix 7. Alteration of the pH and H^+ ion status of droplets on the leaf surface of Artemisia tilesii, Phaseolus vulgaris and Spinacea oleracea.[a]

Rain droplet pH 2.5	Time after droplet application[b]									
	15 min		30 min		45 min		60 min		75 min	
	pH	$[H^+]^c$ ± SD ($X\ 10^{-4}$)	pH	$[H^+]$ ± SD ($X\ 10^{-4}$)	pH	$[H^+]$ ± SD ($X10^{-4}$)	pH	$[H^+]$ ± SD ($X10^{-4}$)	pH	$[H^+]$ ± SD ($X10^{-4}$)
Spinach	2.61	24.6 ± 2.7	2.53	29.3 ± 3.6	2.31	47.8 ± 1.6	2.42	38.2 ± 10.7	2.05	89.5±19.0
Bean	2.52	30.2 ± 1.9	2.52	30.1 ± 4.2	2.33	46.7 ± 9.8	2.27	53.8 ± 21.7	2.03	92.3±27.3
Artemisia (SH[d])	2.59	25.4 ± 4.2	2.55	28.1 ± 5.4	2.47	33.8 ± 6.9	2.45	35.3 ± 15.8	2.20	62.3±27.7
Artemisia (AL[e])	2.52	29.9 ± 1.1	2.54	28.6 ± 7.8	2.38	41.5 ± 5.4	2.52	30.5 ± 13.8	2.05	88.2±28.2
Parafilm (control)	2.47	33.9 ± 1.8	2.40	39.9 ± 3.3	2.26	54.8 ± 4.5	2.13	73.2 ± 5.1	2.03	94.3± 4.9

Continued

Appendix 7 (Continued).

Rain droplet pH 3.0	Time after droplet application[b]									
	15 min		30 min		45 min		60 min		75 min	
	pH	$[H^+]^c$ ± SD (X 10^{-4})	pH	$[H^+]$ ± SD (X 10^{-4})	pH	$[H^+]$ ± SD (X10^{-4})	pH	$[H^+]$ ± SD (X10^{-4})	pH	$[H^+]$ ± SD (X10^{-4})
Spinach	3.30	5.04 ± .65	3.70	1.98 ±1.49	3.12	7.52 ±1.38	3.90	1.27 ± 1.49	2.81	15.50 ±2.11
Bean	3.40	3.94 ± .83	4.52	0.30 ±0.50	3.17	6.70 ±3.53	4.98	0.10 ± 0.13	3.42	3.82 ±4.86
Artemisia (SH[d])	3.61	2.45 ±1.01	3.65	2.21 ±2.00	3.43	3.70 ±2.03	3.63	2.35 ± 2.55	3.86	1.39 ±2.08
Artemisia (AL[e])	3.48	3.29 ± .62	3.89	1.29 ±1.46	3.59	2.56 ±1.99	3.02	9.54 ±18.00	3.14	7.25 ±1.91
Parafilm (control)	3.12	7.48 ± .20	3.06	8.72 ±0.38	3.01	9.80 ±1.38	2.90	12.50 ± 1.39	2.74	18.10 ±0.35

Continued

Appendix 7 (Continued).

Rain droplet pH 3.5	Time after droplet application^b									
	15 min		30 min		45 min		60 min		75 min	
	pH	$[H^+]^c$ ± SD (X 10^{-5})	pH	$[H^+]$ ± SD (X 10^{-6})	pH	$[H^+]$ ± SD (X10^{-6})	pH	$[H^+]$ ± SD (X10^{-6})	pH	$[H^+]$ ± SD (X10^{-5})
Spinach	4.41	3.90 ±4.10	6.74	0.18± 0.06	4.52	30.40±41.20	7.38	0.04± 0.02	4.37	4.22± 5.28
Bean	4.39	4.10 ±2.89	7.24	0.06± 0.02	4.78	16.50±16.00	7.36	0.04± 0.02	4.98	1.04± 0.86
Artemisia (SH^d)	4.60	2.50 ±5.04	6.56	0.28± 0.26	5.32	0.37± 0.53	7.34	0.05± 0.01	4.51	3.12± 6.93
Artemisia (AL^e)	6.25	0.06 ±0.05	7.07	0.08± 0.04	7.02	0.09± 0.05	7.34	0.05± 0.02	5.27	0.54± 1.15
Parafilm (control)	3.66	21.70 ±1.13	3.66	219.0±12.30	3.53	293.0±47.20	3.35	448.0±64.90	3.25	56.80±19.00

Continued

Appendix 7 (Continued).

Rain droplet pH 5.6	Time after droplet application[b]									
	15 min		30 min		45 min		60 min		75 min	
	pH	$[H^+]^c$ ± SD (X 10^{-7})	pH	$[H^+]$ ± SD (X 10^{-7})	pH	$[H^+]$ ± SD (X10^{-7})	pH	$[H^+]$ ± SD (X10^{-7})	pH	$[H^+]$ ± SD (X10^{-7})
Spinach	6.77	1.69± 0.32	7.06	0.88± 0.26	7.08	0.83± 0.24	6.99	1.02± 0.37	6.77	1.71± 0.48
Bean	6.89	1.28± 0.20	7.07	0.85± 0.52	6.86	1.38± 0.30	7.02	0.95± 0.41	6.77	1.69± 1.11
Artemisia (SH[d])	7.08	0.83± 0.22	7.13	0.75± 0.33	6.94	1.15± 0.53	7.14	0.73± 0.24	6.84	1.45± 0.52
Artemisia (AL[e])	7.07	0.84± 0.56	6.96	1.09± 0.36	6.97	1.07± 0.27	6.90	1.25± 1.12	6.45	3.51± 1.18
Parafilm (control)	5.46	34.10±14.40	5.46	34.20±11.70	5.56	27.70± 4.43	5.59	25.80± 9.30	5.38	41.20± 5.92

[a] Values are the mean of 5 leaf replication and on each leaf 3 drops were measured.

[b] Leaves of plants were randomly selected for the droplet pH measurements taken through time.

[c] Hydrogen ion concentrations, are in μeq l^{-1}.

[d] SH = Smoking Hills #11

[e] AL = Alaska #3

Appendix 8. The effect of leaf age on the ability to neutralize acidic (pH 3.0) droplets for leaves of Spinacea oleracea.

	Mean pH and hydrogen ion concentration[a] of droplets on the leaf									
	Leaf 1 (youngest)		Leaf 2		Leaf 3		Leaf 4		Leaf 5 (oldest)	
Time[b] (mins)	pH	$[H^+]$ ± SD ($X\ 10^{-4}$)	pH	$[H^+]$ ± SD ($X\ 10^{-4}$)	pH	$[H^+]$ ± SD ($X10^{-4}$)	pH	$[H^+]$ ± SD ($X10^{-4}$)	pH	$[H^+]$ ± SD ($X10^{-5}$)
15	3.61	2.45± 0.63	3.76	1.75± 1.13	3.70	1.99± 2.16	4.09	0.82± 0.87	3.94	11.50±14.20
30	3.72	1.91± 1.18	3.53	2.98± 1.92	3.54	2.91± 2.31	4.48	0.33± 0.52	5.97	0.11± 0.12
45	3.39	4.04± 2.17	3.56	2.73± 2.75	3.39	4.04± 3.52	3.88	1.32± 1.41	4.44	3.59± 7.93
60	3.36	4.37± 2.00	3.40	3.97± 3.68	3.40	3.94± 3.34	3.99	1.03± 1.69	5.02	0.96± 1.90
75	3.48	3.28± 4.28	3.18	6.55± 5.24	3.29	5.16± 6.64	4.21	0.61± 1.34	5.27	0.54± 1.10

[a] Hydrogen ion concentrations are in μeql^{-1}.